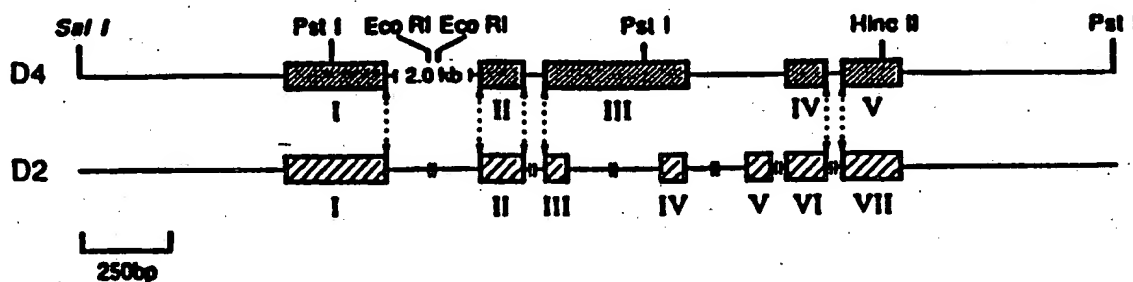




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(54) Title: A NOVEL HUMAN DOPAMINE RECEPTOR AND ITS USES



(57) Abstract

The present invention is directed toward the isolation, characterization and pharmacological use of the human D4 dopamine receptor. The nucleotide sequence of the gene corresponding to this receptor and allelic variant thereof are provided by the invention. The invention also includes recombinant eukaryotic expression constructs capable of expressing the human D4 dopamine receptor in cultures of transformed eukaryotic cells. The invention provides cultures of transformed eukaryotic cells which synthesize the human D4 dopamine receptor, and methods for characterizing novel psychotropic compounds using such cultures.

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A NOVEL HUMAN DOPAMINE RECEPTOR AND ITS USES

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. Patent Application Serial No. 07/626,618, filed on December 7, 1990, which is hereby incorporated by reference.

This invention was made with government support under NIMH grant MH-45614 awarded by the National Institutes of Health, United States of America, and grant PG 11121 awarded by the Medical Research Council of Canada. The governments have certain rights in the invention.

1. Field of the Invention

The invention relates to dopamine receptors from mammalian species and the genes corresponding to such receptors. In particular, it relates to the human dopamine receptor D4. Specifically, the invention relates to the isolation, cloning and sequencing of the human D4 receptor gene. The invention also relates to the construction of eukaryotic expression vectors capable of expression of the human D4 dopamine receptor in cultures of transformed eukaryotic cells and the synthesis of the human D4 dopamine receptor in such cultures. The invention relates to the use of such cultures of transformed eukaryotic cells producing the human D4 dopamine receptor for the characterization of antipsychotic drugs.

2. Information Disclosure Statement

Dopamine is a neurotransmitter that participates in a variety of different functions mediated by the nervous system, including vision, movement, and behavior (*see generally* Cooper *et al.*, 1978, The Biochemical Basis of Neuropharmacology, 3d ed., Oxford University Press, New York, pp. 161-195). The diverse physiological actions of dopamine are in turn mediated by its interaction with two of the basic types of G protein-coupled receptors, D1 and D2, which respectively stimulate and inhibit the enzyme adenylyl cyclase (Kebabian & Calne, 1979, *Nature* 277: 93-96). Alterations in the number or activity of these receptors may be a contributory factor in disease states such as

Parkinson's disease (a movement disorder) and schizophrenia (a behavioral disorder).

5 A great deal of information has accumulated on the biochemistry of the D1 and D2 dopamine receptors, and methods have been developed to solubilize and purify these receptor proteins (*see* Senogles *et al.*, 1986, *Biochemistry* 25: 749-753; Sengoles *et al.*, 1988, *J. Biol. Chem.* 263: 18996-19002; Gingrich *et al.*, 1988, *Biochemistry* 27: 3907-3912). The D1 dopamine receptor in several tissues appears to be a glycosylated membrane protein of about 72 kD (Amlaiky *et al.*, 1987, *Mol. Pharmacol.* 31: 129-134; Ninik *et al.*, 1988, *Biochemistry* 27: 7594-7599). The D2 receptor has been suggested to have a higher molecular weight of about 90 - 150 kD (Amlaiky & Caron, 1985, *J. Biol. Chem.* 260: 1983-1986; Amlaiky & Caron, 1986, *J. Neurochem.* 47: 196-204; Jarvie *et al.*, 1988, *Mol. Pharmacol.* 34: 91-97). Much less is known about a recently discovered additional dopamine receptor, termed D3 (Sokoloff *et al.*, 1990, *Nature* 347: 146-151).

15 Dopamine receptors are primary targets in the clinical treatment of psychomotor disorders such as Parkinson's disease and affective disorders such as schizophrenia (Seeman *et al.*, 1987, *Neuropsychopharm.* 1: 5-15; Seeman, 1987, *Synapse* 1: 152-333). The three different dopamine receptors (D1, D2, D3) have been cloned as a result of nucleotide sequence homology which exists between these receptor genes (Bunzow *et al.*, 1988, *Nature* 336: 783-787; Grandy *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86: 9762-9766; Dal Toso *et al.*, 1989, *EMBO J.* 8: 4025-4034; Zhou *et al.*, 1990, *Nature* 346: 76-80; Sunahara *et al.*, 1990, *Nature* 346: 80-83; Sokoloff *et al.*, 1990, *Nature* 347: 146-151).

25 The antipsychotic clozapine is useful for socially withdrawn and treatment-resistant schizophrenics (*see* Kane *et al.*, 1990, *Nature* 347: 146-151), but unlike other antipsychotic drugs, clozapine does not cause tardive dyskinesia (*see* Casey, 1989, *Psychopharmacology* 99: 547-553). Clozapine, however, has dissociation constants for D2 and D3 which are 3 to 30-fold higher than the therapeutic free concentration of clozapine in plasma water (Ackenheil *et al.*, 1976, *Arzneim-Forsch* 26: 1156-1158; Sandoz Canada, Inc., 1990, *Clozaril*: Summary of

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preclinical and clinical data). This suggests the existence of dopamine receptors more sensitive to the antipsychotic clozapine than those known in the prior art heretofore.

5 We have cloned and sequenced such a human dopamine receptor which we term D4. The dopamine D4 receptor gene has high homology to the human dopamine D2 and D3 receptor genes. The pharmacological profile of this receptor resembles that of the D2 and D3 receptors but it has an affinity for clozapine which is tenfold higher. The present inventors envision that the D4 dopamine receptor disclosed as this invention may prove useful in discovering
10 new types of drugs for schizophrenia that like clozapine do not induce tardive dyskinesia and other motor side effects.

We have also discovered that the D4 gene is polymorphic in the human population, having at least 7 different alleles that can be detected by restriction fragment length polymorphism analysis (*see*, Botstein *et al.*, 1980, Am. J. Hum. Genet. 32: 314-331). This is the first receptor in the catecholamine receptor
15 family which displays polymorphic variations in the human population. The observed polymorphism in dopamine D4 receptor genes may underlie individual differences in susceptibility to neuropsychiatric disorders such as schizophrenia and manic depression, as well as responsiveness to antipsychotic medication.

20

SUMMARY OF THE INVENTION

The present invention is directed toward the isolation, characterization and pharmacological use of the human D4 dopamine receptor, the gene corresponding to this receptor, a recombinant eukaryotic expression construct capable of
25 expressing the human D4 dopamine receptor in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the human D4 dopamine receptor.

It is an object of the invention to provide a nucleotide sequence encoding a mammalian dopamine receptor. Further, it is an object of the invention to
30 provide a nucleotide sequence that encodes a mammalian dopamine receptor with novel and distinct pharmacological properties. It is specifically an object of the

invention to provide a nucleotide sequence encoding a mammalian dopamine receptor having the particular drug dissociation properties of the human dopamine receptor D4. In particular, the mammalian dopamine receptor encoded by the nucleotide sequence of the present invention has a high affinity for the drug
5 clozapine. The human D4 dopamine receptor embodied in the present invention shows a dissociation constant (termed K_d) of 1-40 nanomolar (nM), preferably 1-20 nM, most preferably 11 nM clozapine, as detected by the [3 H]spiperone binding assay disclosed herein. The human D4 dopamine receptor embodied in the present invention displays the following pharmacological profile of inhibition
10 of [3 H]spiperone binding in the [3 H]spiperone binding assay: spiperone > eticlopride > clozapine > (+)-butaclamol > raclopride > SCH23390. In a preferred embodiment of the invention, the nucleotide sequence encoding a dopamine receptor encodes the human dopamine receptor D4.

The present invention provides a nucleotide sequence encoding a
15 mammalian dopamine receptor that is the human D4 receptor. In a preferred embodiment, this nucleotide sequence comprises a cDNA sequence isolated from RNA derived from the human neuroblastoma cell line SK-N-MC [SEQ ID No: 17], comprising the sequences of the D4.2 allele of the human D4 dopamine receptor gene. In another preferred embodiment, this nucleotide sequence
20 comprises a cDNA sequence isolated from RNA derived from human pituitary gland tissue [SEQ ID No: 19]. In yet another preferred embodiment, this nucleotide sequence comprises a cDNA sequence isolated from RNA derived from human substantia nigra tissue [SEQ ID No.: 19]. Both of these embodiments
25 comprise the sequences of the D4.4 allele of the human D4 dopamine receptor gene.

The invention also includes a nucleotide sequence derived from human
genomic DNA [SEQ ID Nos.: 1,3,4,5,7,12,14 & 15] comprising the sequences
of the D4.7 allele of the human D4 dopamine receptor gene, and a nucleotide
sequence derived from human genomic DNA [SEQ ID Nos.: 1,3,4,5,7,10,14 &
30 15] comprising the sequences of the D4.4 allele of the human D4 dopamine receptor gene. In this embodiment of the invention, the nucleotide sequence

includes 5 kilobases (kb) of human genomic DNA encoding the dopamine receptor D4. This embodiment includes the sequences present in the cDNA embodiments as well as nucleotide sequences of 5' untranslated sequence, three intervening sequences that interrupt the coding sequence of the human D4 dopamine receptor gene, and 3' untranslated sequences. Also provided is a cDNA sequence derived from the genomic DNA sequence of the D4.4. allele [SEQ ID No: 19] and the D4.7 allele [SEQ ID No: 21] of the human D4 dopamine receptor gene.

The invention includes a nucleotide sequence of a human D4 receptor molecule, and includes allelic variations of this nucleotide sequence and the corresponding D4 receptor molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, having essentially the same nucleotide sequence as the nucleotide sequence of the human D4 receptor disclosed herein, wherein the resulting human D4 receptor molecule has substantially the same drug dissociation properties of the human D4 receptor molecule corresponding to the nucleotide sequence described herein. Specific preferred embodiments include alleles D4.2, D4.4 and D4.7 of the human D4 dopamine receptor gene, as defined herein.

The invention provides sequences of the naturally-occurring alleles of the human D4 dopamine receptor gene. Such alleles are defined as comprising from about 2 to about 8 repeats of a nucleotide sequence that is substantially homologous to the sequence [SEQ ID Nos: 8,10,12,17,19,21]:
5'-A CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC-3'.

Allelic variations of this nucleotide sequence and the corresponding D4 receptor molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, having essentially the same nucleotide sequence as the nucleotide sequence of the human D4 receptor disclosed herein, wherein the resulting human D4 receptor molecule has substantially the same drug dissociation properties of the human D4 receptor molecule corresponding to the nucleotide sequence described herein are additional preferred embodiments of the invention. Specific preferred embodiments include the allele D4.2, comprising 2 copies of

the repeat tandemly repeated [SEQ ID Nos: 8 & 17]; the allele D4.4, comprising 4 copies of the repeat tandemly repeated [SEQ ID Nos: 10 & 19]; and the allele D4.7, comprising 7 copies of the repeat tandemly repeated [SEQ ID Nos: 12 & 21].

5 The invention also includes a predicted amino acid sequence for the human D4 dopamine receptor deduced from the nucleotide sequence comprising the complete coding sequence of the D4 dopamine receptor gene [SEQ ID Nos: 18, 20 & 22]. Specific preferred embodiments comprise the amino acid sequence of the naturally-occurring alleles of the human D4 dopamine receptor gene. Such
10 alleles are defined as comprising from about 2 to about 8 repeats of an amino acid sequence that is substantially homologous to the sequence [SEQ ID Nos: 9,11,13,18,20,22]:

(P/A)AP(R/G)LP(Q/R/P)(D/G)PCG(P/S)(D/N)CAP

15 Allelic variations of this amino acid and the corresponding D4 receptor molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, having essentially the same amino acid sequence as the human D4 receptor disclosed herein, wherein the human D4 receptor molecule has substantially the same drug dissociation properties of the human D4 receptor molecule corresponding to the amino acid sequence described herein are additional
20 preferred embodiments of the invention. Specific preferred embodiments include the allele D4.2, comprising 2 copies of the repeat tandemly repeated [SEQ ID Nos: 9 & 18]; the allele D4.4, comprising 4 copies of the repeat tandemly repeated [SEQ ID Nos: 11 & 20]; and the allele D4.7, comprising 7 copies of the repeat tandemly repeated [SEQ ID Nos: 13 & 22].

25 This invention provides both nucleotide and amino acid probes derived from these sequences. The invention includes probes isolated from either cDNA or genomic DNA clones, as well as probes made synthetically with the sequence information derived therefrom. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or *in vitro* amplified
30 probes made using cDNA or genomic clones embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the

nucleotide sequence information of cDNA or genomic clone embodiments of the invention. The sequence information provided by the present invention is also intended to provide the basis for *in vitro* amplification methods for detecting D4 dopamine receptor alleles comprising the genotype of somatic and germ cells, zygotes, embryos, and tissues in humans and other mammals for diagnostic, therapeutic and other purposes.

It is a further object of this invention to provide sequences of the human D4 dopamine receptor for use as probes to determine the pattern, amount and extent of expression of this receptor in various tissues of mammals, including humans. It is also an object of the present invention to provide probes derived from the sequences of the human D4 dopamine receptor to be used for the detection and diagnosis of genetic diseases. It is an object of this invention to provide probes derived from the sequences of the human D4 dopamine receptor to be used for the detection of novel related receptor genes.

The present invention also includes synthetic peptides made using the nucleotide sequence information comprising the cDNA or genomic clone embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of D4 dopamine receptor-specific antibodies, or used for competitors of the D4 receptor molecule for drug binding, or to be used for the production of inhibitors (or blockers) of the binding of dopamine or dopamine analogs of the D4 dopamine receptor molecule. As used herein, the term "inhibitor of dopamine binding" is intended to encompass biochemical agonists and/or antagonists of dopamine binding to the D4 dopamine receptor.

In addition, this invention includes recombinant DNA constructs comprising the human D4 dopamine receptor and sequences that mediate the replication and selected growth of microorganisms that carry this construct.

The present invention provides recombinant expression constructs comprising the nucleotide sequence of the human D4 dopamine receptor and sequences sufficient to direct the synthesis of the human D4 dopamine receptor protein in cultures of transformed eukaryotic cells. In preferred embodiments, the

recombinant expression construct is comprised of plasmid sequences derived from the plasmid pCD-PS and D4 dopamine receptor sequences corresponding to cDNA sequences for alleles D4.2, D4.4 and D4.7, as defined herein, as well as a hybrid human D4 dopamine gene, comprised of the entirety of the genomic sequences from a particular D4 dopamine genomic clone described herein, up to a *Pst*I site located in exon III, followed by the remainder of the coding and 3' untranslated sequences found in a particular human cDNA sequence derived from a human neuroblastoma cell line. Recombinant expression constructs of the invention also encompass embodiments comprising allelic variations of the human D4 dopamine receptor genomic DNA sequences and cDNA-derived sequences. This invention includes recombinant expression constructs comprising essentially the nucleotide sequences of genomic and cDNA clones of the human D4 dopamine receptor and allelic variations thereof in embodiments that provide for the expression of human D4 dopamine receptor protein in cultures of transformed eukaryotic cells.

It is also an object of this invention to provide cultures of transformed eukaryotic cells that have been transformed with such recombinant expression constructs and that synthesize human D4 dopamine receptor protein. In a preferred embodiment, the invention provides monkey COS cells that synthesize human D4 dopamine receptor protein.

The present invention also includes protein preparations of the human D4 dopamine receptor, and preparations of membranes containing the human D4 dopamine receptor, derived from cultures of eukaryotic cells transformed with the recombinant expression constructs of the invention. In a preferred embodiment, cell membranes containing human D4 dopamine receptor protein are isolated from culture of COS-7 cells transformed with a recombinant expression construct that directs the synthesis of human D4 dopamine receptor.

It also an object of this invention to provide the human D4 dopamine receptor for use in the *in vitro* screening of novel antipsychotic compounds. In a preferred embodiment, membrane preparations containing the human D4 dopamine receptor, derived from cultures of eukaryotic cells transformed with the recombinant expression constructs of the invention, are used to determine the drug

dissociation properties of antipsychotic compounds *in vitro*. These properties are then used to characterize novel antipsychotic compounds by comparison to the binding properties of known antipsychotic compounds.

5 The present invention will also be useful for the detection of dopamine and dopamine analogues, known or unknown, either naturally occurring or as the embodiments of antipsychotic or other drugs.

10 It is an object of the present invention to provide a method for the quantitative detection of dopamine and dopamine analogues, either naturally occurring or as the embodiments of antipsychotic or other drugs. It is an additional object of the invention to provide a method to detect dopamine or dopamine analogues in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, or any other bodily fluid.

DESCRIPTION OF THE DRAWINGS

15 Figure 1 illustrates the structure of a genomic clone comprising the human D4 dopamine receptor gene.

Figure 2 illustrates the nucleotide sequence of genomic and cDNA clones of the human D4 dopamine receptor gene.

20 Figure 3 provides an amino acid sequence alignment of mammalian dopamine receptors

Figure 4 shows the binding of [³H]spiperone to membranes of COS-7 cell transfected with a recombinant expression construct that expresses the human D4 receptor protein.

25 Figure 5 demonstrates the pharmacological specificity of [³H]spiperone binding to COS-7 cells transfected with a human D4 receptor expression construct.

Figure 6 illustrates the structure of a genomic clone comprising the human D4 dopamine receptor gene and the nucleic acid and corresponding amino acid sequences of 2, 4 and 7 copies of a novel 48 bp tandem repeat.

30 Figure 7 illustrates restriction fragment length polymorphic variants of the human D4 receptor gene in 9 individuals.

Figure 8 demonstrates the transcriptional integrity of each of three cloned variant human D4 receptor gene expression constructs expressed in transfected COS-7 cells.

Figure 9 illustrates Scatchard analysis (panels a) and [³H]-spiperone competition binding experiments (panels b) of each of three cloned variant human
5 D4 receptor gene expression constructs expressed in transfected COS-7 cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "D4 dopamine receptor" as used herein refers to proteins
10 substantially homologous to, and having substantially the same biological activity as, the protein coded for by the nucleotide sequences depicted in Figure 2 and Figure 6 (i.e., proteins which display high affinity binding to clozapine) [SEQ ID Nos: 1,3,4,5,7,8,10,12,14 & 15]. This definition is intended to encompass natural allelic variations in the D4 dopamine receptor sequence, specifically
15 including the alleles D4.2, D4.4 and D4.7, as defined herein [SEQ ID Nos.: 17,19 & 21], and all references to the D4 dopamine receptor, and nucleotide and amino acid sequences thereof are intended to encompass such allelic variations, both naturally-occurring and man-made. Cloned genes of the present invention may code for D4 dopamine receptors of any species of origin, including, mouse,
20 rat, rabbit, cat, and human, but preferably code for receptors of mammalian, most preferably human, origin.

The production of proteins such as the D4 dopamine receptor from cloned genes by genetic engineering is well known (*see, e.g.*, U.S. Patent No. 4,761,371 to Bell *et al.* at Col. 6 line 3 to Col. 9 line 65; the disclosure of all U.S. patent
25 references cited herein is to be incorporated herein by reference). The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

DNA which encodes the D4 dopamine receptor may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts
30 of mRNA from appropriate tissues, cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures,

as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the D4 dopamine receptor gene sequence information provided herein. Probes may be labeled with a detectable group in accordance with know procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, D4 dopamine receptor gene sequences may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the D4-dopamine receptor gene sequence provided herein (see U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis).

The D4 dopamine receptor may be synthesized in host cells transformed with constructs containing DNA encoding the D4 dopamine receptor. Such constructs are replicable and are used herein either to amplify DNA encoding the D4 dopamine receptor and/or to express DNA which encodes the D4 dopamine receptor. An expression construct is a replicable DNA construct in which a DNA sequence encoding the D4 receptor is operably linked to suitable control sequences capable of effecting the expression of the D4 receptor in a suitable host. The need for such control sequences will vary depending upon the host selected and the transfection method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. When used for DNA amplification such constructs do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selective marker gene to facilitate recognition of transformants.

Constructs useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The construct may replicate and function independently of the host genome, or may, in some instances, integrate into the host genome itself. Suitable constructs will contain replicon and control sequences which are derived from species compatible

with the intended expression host. Transformed host cells are cells which have been transformed, transfected or infected with the D4 receptor-containing constructs assembled using recombinant DNA techniques. Transformed host cells ordinarily express the D4 receptor, but host cells transformed for purposes of cloning or amplifying the D4 receptor DNA need not express the D4 receptor. When expressed, the D4 receptor will typically be located in the host cell membrane.

DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leaders sequences, contiguous and in the same translational reading frame.

Cultures of cells derived from multicellular organisms are a desirable host for recombinant D4 dopamine receptor synthesis. In principal, any higher eukaryotic cell culture can be used, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure (*see Tissue Culture*, Academic Press: New York (Kruse & Patterson, eds.) 1973). Examples of useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI138, BHK, COS-7, CV, and MDCK cell lines. Expression constructs for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream from the gene to be expressed, along with a ribosome binding site, RNA splice site (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

The transcriptional and translational control sequences in expression constructs to be used in transforming vertebrate cells are often provided by viral sources. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and Simian Virus 40 (SV40; *see, e.g.*, U.S. Patent No. 4,599,308). The early and late promoters of SV40 are useful because both are obtained easily from the virus within a fragment which also contains the SV40

viral origin of replication (see Fiers *et al.*, 1978, Nature 273: 113). Further, the human genomic D4 receptor promoter, control and/or signal sequences, may also be used, provided such control sequences are compatible with the host cell chosen.

5 An origin of replication may be provided either within the construct itself, such as may be derived from SV40 or other viral source (e.g., Polyoma, Adenovirus, VSV, or MPV), or may be provided by the host cell chromosomal replication mechanism. If the construct is integrated into the host cell chromosome, the latter may be sufficient.

10 D4 dopamine receptors made from cloned genes in accordance with the present invention may be used for screening compounds for D4 dopamine receptor activity, or for determining the amount of a dopaminergic drug in a solution (e.g., blood plasma or serum). For example, host cells may be transformed with a construct of the present invention, D4 dopamine receptors expressed in that host,
15 the cells lysed, and the membranes from those cells used to screen compounds for D4 dopamine receptor binding activity. Competitive binding assays in which such procedures may be carried out are well known, as illustrated by the Examples below. By selection of host cells which do not ordinarily express a dopamine receptor, pure preparations of membranes containing D4 receptors can be
20 obtained. Further, D4 dopamine receptor agonist and antagonists can be identified by transforming host cells with constructs of the present invention. Membranes obtained from such cells can be used in binding studies wherein the drug dissociation constants are measured. Such cells must contain D4 protein in the plasma and other cell membranes. Procedures for carrying out assays such
25 as these are also described in greater detail in the Examples which follow.

 Cloned genes and constructs of the present invention are useful to transform cells which do not ordinarily express the D4 dopamine receptor to thereafter express this receptor. Such cells are useful as intermediates for making cell membrane preparations for receptor binding assays, which are in turn useful
30 for drug screening. Further, genes and constructs of the present invention are useful in gene therapy. For such purposes, retroviral constructs as described in

U.S. Patent No. 4,650,764 to Temin and Watanabe or U.S. Patent No. 4,861,719 to Miller may be employed. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out homologous recombination or site-directed mutagenesis (*See generally* Thomas & Capecchi, 1987, Cell 51: 503-512; Bertling, 1987, Bioscience Reports 7: 107112; Smithies *et al.*, 1985, Nature 317: 230-234).

Cloned genes of the present invention, and oligonucleotides derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with genetic polymorphisms within a population. Such RFLPs may also be associated with certain genetic disorders, and the probes provided by the invention can be used for their identification and the identification of individuals susceptible to neuropsychiatric disorders such as schizophrenia and manic depression. Such RFLPs may also be useful for predicting individual responsiveness to psychotropic and antipsychotic drugs.

Oligonucleotides of the present invention are useful as diagnostic tools for probing D4 receptor gene expression in nervous tissue. For example, tissue can be probed *in situ* with oligonucleotide probes carrying detectable label groups by conventional autoradiography techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the location of the D4 dopamine receptor gene, and potential pathological conditions related thereto, as also illustrated by the Examples below.

Oligonucleotides of the present invention are also useful for *in vitro* amplification of D4 dopamine receptor sequences. Amplification methods include but are not intended to be limited to the polymerase chain reaction and the ligase chain reaction. Amplification of D4 dopamine receptor sequences is useful as a diagnostic tools for analyzing and quantitating D4 receptor gene expression in tissue, for example nervous tissue. Additionally, the use of oligonucleotides synthesized or isolated according to methods well known in the art that comprise D4 dopamine receptor sequences provided by the invention permit *in vitro* amplification methods to be used for the detection of D4 dopamine receptor alleles

comprising the genotype of somatic and germ cells, zygotes, embryos, and tissues in humans and other mammals for diagnostic, therapeutic and other purposes.

5 The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Screening Tissue and Cell Line RNA for 10 Dopamine Receptor Expression

RNA was prepared from different rat tissues or cell lines using the guadinium thiocyanate/CsCl procedure described in Bunzow *et al.*, 1988, Nature 336: 783-787. Tissues tested included heart, epididymis, testis, gut, pancreas, spleen, thymus, muscle, ventricle, atria, lung, adrenal, kidney, liver, pineal gland and pituitary. Cell lines screened included SK-N-MC, SK-N-SH, COS, AKR1, 15 Ltk, GH4C1, NG108-15, AtT20, 3T3, BSC40, C6, CV-1, Hela, IMR-32, N4TG1, NCB-20, PC-12, Rin m5f and WERI-Rb-1. 20 µg of RNA was analyzed by Northern blot hybridization with a radiolabeled *Bsr*YI-*Bgl*III DNA fragment of the rat D2 receptor, which encodes the putative transmembrane domains VI and 20 VII. Blots were hybridized under standard conditions as described in Bunzow *et al.*, *ibid.*; hybridization was performed overnight at 37°C. Blots were then washed at 55°C in 2X standard saline-citrate (SSC) and 1% sodium dodecyl sulfate (SDS). Washed blotes were exposeed to X-ray film for two days at -70°C using an intensifying screen. For comparison, the same blot was hybridized under 25 high stringency conditions (the modifications of which include using 50% formamide and 42°C for the hybridication and 0.2X SSC for the wash). Under conditions of low stringency the SK-N-MC cell line showed a positive signal in these experiments.

EXAMPLE 2

30 Construction of a cDNA Phage Library using Neuroblastoma RNA

Double-stranded cDNA was synthesized using standard techniques [see

Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press: New York] from poly(A)⁺ mRNA isolated from the human neuroblastoma cell line SK-N-MC as described in Example 1. The cDNA was directionally cloned into the *EcoRI* and *XhoI* restriction endonuclease sites of the phage cloning vector lambda ZAPII (Stratagene, La Jolla, CA). The library was transferred to colony plaque screen filters (New England Nuclear, Boston, MA). Approximately 500,000 independent clones were screened under low-stringency hybridization conditions as described in Example 1. Hybridization was performed for 30 hrs with ³²P-labeled 1.6 kb *Bam*HI - *Bgl*II and 300 bp *Bsr*YI - *Bgl*II fragments of a rat D2 receptor clone at a specific activity of 10⁶ dpm/μg. Filters were washed at 55°C in 2X SSC and 1% SDS. The clone D210S was isolated and sequenced using the Sanger dideoxy chain termination method catalyzed by Sequenase (U.S. Biochemical Corporation, Cleveland, OH). The sequence of this clone is shown in Figure 2 (hatched area).

The putative coding sequence is shown in capitals (non-coding sequence is in italics) and the deduced amino acid sequence is shown above the nucleotide sequence. Numbering of the putative coding sequence begins with the first methionine of the open reading frame. The sequence corresponding to the cDNA clone is hatched. Single-letter abbreviations for amino acids and nucleotides used herein can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33. Noteworthy is the presence of a duplicated 48 bp sequence in the putative third exon, corresponding to the third cytoplasmic loop region of the D4 receptor protein. The complete nucleotide sequence of this clone has been determined (see Figure 6, wherein these repeated sequences of this clone are designated D4.2 [SEQ ID No: 17]).

EXAMPLE 3

Screening a Genomic DNA Phage Library with a Human Dopamine Receptor Probe

Clone D210S was ³²P-labeled by random primed synthesis and used to screen a commercially available human genomic library cloned in the phage vector EMBL3 (Clontech, Palo Alto, CA). Hybridization was performed as

described in Example 2 using 50% formamide. After hybridization the filters were washed at 65°C in 0.1X SSC and 0.1% SDS. The clone D210G was isolated and analyzed by restriction endonuclease and Southern blot analysis. The map of this genomic clone is shown in Figure 1, wherein the structure of the D4 receptor gene is compared with the structure of the D2 gene. Relevant restriction endonuclease sites in the D4 receptor sequence are indicated. The *SaII* site is part of the cloning site in EMBL3. The proposed coding regions are boxed and numbered in Roman numerals. Perfect matches of proposed intron/exon junction sites are indicated by connecting stippled bars between the receptor clones.

PstI - *PstI* fragments of approximately 1.3 kb and 2.6 kb, and an overlapping *SaII* - *EcoRI* fragment of approximately 2.0 kb derived from the D4 receptor gene were subcloned into the plasmid pBluescript-SK (Stratagene). The subcloned fragments were characterized by sequence analysis as described above. This sequence is shown in Figure 2. The complete nucleotide sequence of this clone has been determined (see Figure 6, wherein these repeated sequences of this clone are designated D4.7 [SEQ ID No: 21]).

EXAMPLE 4

DNA Sequence Analysis of the Human D4 Dopamine Receptor

One of the cDNA clones detected by screening the SK-N-MC neuroblastoma library with a rat D2 probe at low stringency (D210S) contained a 780 bp *EcoRI-XhoI* insert which hybridized to the rat probe. Screening of a human genomic EMBL3 library (Clontech) under high stringency conditions with the clone D210S resulted in the isolation of the genomic clone D210G.

Southern blot and sequence analysis indicated that the clone contained a 5 kb *SaII-PstI* fragment which coded for the entire gene of D210S [SEQ ID No.: 21]. Sequence analysis of this insert showed the presence of an open reading frame with homology to the amino acid sequence of transmembrane domains V (45%), VI (46%) and VII (78%) of the D2 receptor, shown in Figure 3. The putative amino acid sequence of the human D4 receptor [SEQ ID No.: 22] is aligned with the human and rat D2, rat D3 and human and rat D1 receptor

sequences. Amino acids conserved within the group of dopamine receptors are shaded. The putative transmembrane domains are overlined and labeled by Roman numerals.

5 There is a potential translation initiation codon (ATG) 590 bp downstream from the *SaII* site, followed by an open reading frame that showed amino acid sequence homology with transmembrane domain I (36%) and II (63%) of the D2 receptor. Almost immediately downstream from the transmembrane domain II sequence, homology to the D2 receptor disappears, indicating the presence of an intron in the genomic DNA. This intron spanned approximately 2 kb, after which
10 sequence homology to the D2 receptor was re-established. Translation of the putative gene product showed homology to the transmembrane domains III (68%), IV (37%), V(46%) and VII (78%) of the D2 receptor (see Figure 3).

Potential splice junction donor and acceptor sites (Mount, 1982, Nucl. Acids Res. 10: 461-472) were found in the transmembrane domains II, III and VI,
15 as shown in Figure 1. These splice sites were at an identical position as in the D2 and D3 receptor gene [see Grandy *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86: 9762-9766; Dal Toso *et al.*, 1989, EMBO J. 8: 4025-4034; Sokoloff *et al.*, 1990, Nature 347: 146-151] and Figure 1. The coding sequence downstream from transmembrane domain IV is identical to the sequence of clone D210S but
20 is interrupted by an intron of about 300 bp between transmembrane domains V and VI and an additional intron of 92 bp in transmembrane VI (Figure 1, hatched area). The precise location of the splice site for the intron between transmembrane V and VI cannot be determined due to the fact that a sequence of 52 bp present in the coding sequence is repeated exactly on either side of the
25 intron (Figure 2).

The deduced amino acid sequence from the genomic and cDNA nucleotide sequences indicated that this gene codes for a protein of 387 amino acids with an apparent molecular weight of 41kD. A hydrophobicity plot of the protein sequence suggests the existence of seven transmembrane domains. These regions
30 correlate with the observed homologous regions in the human D2 receptor and other receptors belonging to the family of G-protein coupled receptors (Dohlman

et al., 1987, *Biochemistry* 26: 2657-2664; Bunzow *et al.*, 1988, *Nature* 336: 783-787; Sokoloff *et al.*, 1990, *Nature* 347: 146-151; and Figure 2). A potential N-linked glycosylation site (Hubbard & Ivatt, 1981, *Ann. Rev. Biochem.* 50: 555-583) is located two amino acids downstream from the initiation methionine. The amino acid residues Asp (80) and Asp (115) in the D4 receptor, which are conserved within the family catecholaminergic receptors, are postulated to act as "counterions" in catecholamine binding (Strader *et al.*, 1988, *J. Biol. Chem.* 263: 10267-10271). Also conserved within the family of catecholaminergic receptors are Ser (197) and Ser (700) which have been suggested to interact with the catechol hydroxyl groups (Kozak, 1984, *Nucleic Acids Res.* 12: 857-872). Several consensus sites for potential phosphorylation by protein kinase C and protein kinase A are found in the third cytoplasmic loop (Sibley *et al.*, 1987, *Cell* 48: 913-922; Bouvier *et al.*, 1988, *Nature* 333: 370-373). The Cys (187), which may serve as a substrate for palmitoylation, is conserved in most of the G-protein coupled receptors (O'Dowd *et al.*, 1989, *J. Biol. Chem.* 264: 7564-7569). The short carboxyl tail, which terminates similar to the D2 and D3 receptor at Cys (387) (Bunzow *et al.*, 1988, *Nature* 336: 783-787; Grandy *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86: 9762-9766; Dal Toso *et al.*, 1989, *EMBO J.* 8: 4025-4034; Sokoloff *et al.*, 1990, *Nature* 347: 146-151), and the relatively large third cytoplasmic loop, are features observed in most receptors which interact with an isoform of the G protein.

A noteworthy feature of the sequence of the third exon of the genomic D4 receptor clone is the presence of a 7-fold repeat of a GC rich, 48 bp sequence, beginning at nucleotide 447 of exon III, and encodes a proline-rich portion of the D4 dopamine receptor protein (*see* Figure 6, wherein these sequences of this clone are designated D4.7 [SEQ ID No.:21]). This region of the protein corresponds to the putative third cytoplasmic loop of the receptor protein molecule [SEQ ID No.: 22]. This sequence corresponds to the 2-fold repeat of a homologous sequence found in the SK-N-MC neuroblastoma cDNA sequence described in Example 2, suggesting that the D4 receptor gene may be polymorphic. This sequence is uniquely found in the D4 receptor and is not

homologous to any other known dopamine receptor protein. Interestingly, this region of the human D4 receptor is not found in the rat homologue of the D4 receptor, making this variation specific to humans.

From these results we have concluded that the sequences we have isolated
5 encode a polymorphic member of the dopamine receptor family.

EXAMPLE 5

Construction of an Mammalian DNA Expression Construct using Dopamine Receptor cDNA

10 The *ApaI-PstI* gene fragment (Figure 1, the *PstI* site found in exon III after transmembrane domain V) was ligated to the corresponding *PstI-EcoRI* cDNA fragment isolated from the SK-N-MC cDNA. This construct was then cloned into the vector pCD-PS (Bonner *et al.*, 1988, Neuron 1: 403-410). This vector allows for the expression of the human D4 receptor gene from the SV40
15 promoter. Large quantities of the pCD-PS-D4 construct plasmid were prepared using standard techniques (*see*, Sambrook *et al.*, *ibid.*). This plasmid was transfected into COS-7 cells by the calcium phosphate precipitation technique (Gorman *et al.*, 1983, Science 221: 551-553). Two days later membranes cells
20 were harvested and analyzed as described in Example 6.

EXAMPLE 6

Analysis of Dopamine and Dopamine-Antagonist Binding of D4 Dopamine Receptor

Cells were harvested and homogenized using a teflon pestle in 50 mM
25 Tris-HCl (pH 7.4 at 4°C) buffer containing 5 mM EDTA, 1.5 mM CaCl₂, 5 mM MgCl₂, 5 mM KCl and 120 mM NaCl. Homogenates were centrifuged for 15 minutes at 39,000g, and the resulting pellets resuspended in buffer at a concentration of 150-250 µg/ml. For saturation experiments, 0.25 ml aliquots of each tissue homogenate was incubated in duplicate with increasing concentrations
30 of [³H]spiperone (70.3 Ci/mmol; 10-3000 pM final concentration) for 120 min at 22°C in a total volume of 1 ml. The results of these experiments are shown in Figure 4. The results shown are representative of two independent experiments

each conducted in duplicate (the inset show a Scatcherd plot of the same data). Estimated B_{max} (approximately 260 fmol/mg protein) and K_i (70 pM) values were obtained by LIGAND computer program.

5 Representative curves are shown in Figure 5 for the concentration dependent inhibition of [3 H]spiperone binding by various dopaminergic agonist and antagonists. Estimated K_i values are listed in Table I along with the K_i values obtained on the human D2 receptor expressed in GH(4)ZR(7) cells. For competition binding experiments, assays were initiated by the addition of 0.25 ml of membrane preparation and incubated in duplicate with the concentrations of
10 competing ligands indicated in Figure 5 (10^{-14} to 10^{-3} M) and [3 H]spiperone (150-300 pM) for 120 min at 22°C. Assays were terminated by rapid filtration through a Titertek cell harvester and filters subsequently monitored to quantitate radioactive tritium. For all experiments, specific [3 H]spiperone binding was defined as that binding inhibited by 10 μ M (+)sulpiride. Both saturation and
15 competition binding data were analyzed by the non-linear least square curve-fitting program LIGAND run on a Digital Micro-PDP-11. The human D4 dopamine receptor displays the following pharmacological profile of inhibition of [3 H]spiperone binding in this assay: spiperone > eticlopride > clozapine > (+)-butaclamol > raclopride > SCH23390.

20

EXAMPLE 7

Polymorphic Allelic Variants of the D4 Dopamine Receptor Isolated from Human Tissue cDNA Libraries

25 Human cDNA libraries were screened for expression of polymorphic variants of the human D4 receptor gene. A human substantia nigra cDNA library construed in lambda gt11 (Clontech) and a pituitary cDNA library constructed in lambda gt10 as described in Example 2 were screened for clones encoding the D4 receptor. Approximately $0.5-1 \times 10^6$ plaque-forming units (p.f.u.) were
30 transferred in duplicate to nylon filters (DuPont/NEN) and probed with a 32 P-labeled 700 bp *EcoRI-XhoI* fragment encoding the cDNA isolated from the neuroepithelioma SK-N-MC under conditions as described in Example 2 above.

Screening of cDNA libraries from human pituitary and substantia nigra resulted in the isolation of variant cDNA clones of the D4 receptor. The pituitary lambda gt10 clone contained a 1.4-kb *EcoRI* insert, coding for intron 1 and the down-stream sequences of the D4 receptor. This pituitary D4 receptor clone also
5 contained the second intron, but the last intron was spliced out. The isolated substantia nigra lambda gt11 clone contained a 600-bp *EcoRI* insert, coding for the D4 receptor, starting in the 5' site of the putative third cytoplasmic loop. Both these clones contained a four-fold repeat (*see* Figure 6, wherein these sequences of these clones are designated D4.4 [SEQ ID No.: 19]) of the 48-bp
10 sequence previously found as a 7-fold repeat in the D4 genomic clone D210G (Example 4) and a 2-fold repeat in the neuroblastoma SK-N-MC cDNA clone (Example 2) within the putative third cytoplasmic loop of the D4 receptor protein (*compare*, SEQ ID Nos.: 18, 20 & 22]. A comparison of the nucleic acid sequences revealed that, due to the absence of conventional splice junction sites
15 in the seven-fold repeat sequence of the genomic clone, a novel splicing mechanism would be required to account for the existence of the different cDNA clones.

Two different human genomic libraries from different human individuals (Clontech) were screened to detect allelic polymorphism in the human D4 receptor
20 gene. Screening of genomic libraries resulted in the isolation of a genomic clone with a 4-fold repeat of the 48 bp sequence previously detected in pituitary and substantia nigra cDNA. This result indicated that the polymorphic cDNA molecules resulted from genetic polymorphic variation in the corresponding genomic DNA, due to the existence of polymorphic alleles in the human
25 population for the D4 receptor.

EXAMPLE 8

Additional D4 Receptor Gene Allelic Variants Found by RFLP Analysis of Human Genomic DNA

30

The three different D4 receptor sequences predict a restriction fragment length polymorphism for a *HincII-PstI* fragment of the D4 gene (Figure 6).

Southern blot analysis of human genomic DNA was performed as described (see Sambrook *et al.*, *ibid.* and Example 3). A RFLP was observed in humans and the different allelic fragments were sized.

5 Briefly, high molecular weight genomic DNA was isolated from human blood samples using proteinase K and phenol/chloroform extractions. Genomic DNA (5 μ g) was digested with the restriction endonucleases *HincII* and *PstI* and size separated by agarose (1%) gel electrophoresis. DNA was transferred to nylon membranes (Zeta-probe, Biorad) according to standard techniques (Sambrook *et al.*, *ibid.*). Southern blots were probed with a 32 P-labeled 600 bp
10 *EcoRI-HincII* fragment, coding for the D4 cDNA isolated from the neuroepithelioma SK-N-MC, and washed at high stringency (65°C, 0.1xSSC, 0.1% SDS, 40 min). The blot was exposed to X-ray film for three days. Results of these experiments are shown in Figure 7.

The position of a 540-bp size marker is indicated on the left. D4-
15 hybridizing polymorphic bands can be seen at approximately 520 bp, 620 bp, 710 bp, 760 bp and 800 bp. [It will be recognized to those with skill in this art that the sizes given herein for the alleles of the human D4 dopamine receptor gene are limited in their precision to the resolving power of the agarose gels used in the analyses. The sizes are approximate as given herein, and more exact sizes can
20 be calculated from the sequences of the different alleles found in SEQ ID Nos: 17, 19 & 21.] The 520 bp, 620 bp and 760 bp fragments correlate closely with the sizes of the *HincII-PstI* fragments of the cloned D4 receptor variants with the two-, four- and seven-fold repeat sequences respectively. The presence of 710 bp and 800 bp fragments suggests that variants with six-fold and eight-fold repeat
25 sequences also exist. Additional polulation screening experiments have resulted in the detection of alleles corresponding to three-fold and five-fold repeats. A total of 7 alleles of the D4 receptor gene have accordingly been found in the human population.

EXAMPLE 9

Expression of Allelic Variants of the D4 Receptor

Mammalian DNA expression constructs were made as described in Example 5 for expression of the allelic variants of the D4 receptor. Various cDNA constructs were cloned into the expression vector pCD-PS (see Example 5) which contains the SV40 origin of replication and drives expression of the cloned inserts from the SV40 late promoter. A 1.7-kb *KpnI-XbaI* fragment comprising a cDNA for the D4 receptor gene containing the 7-fold repeat was cloned into the pCD-PS vector of Example 5 and called hereafter pCD-D4.7. Full-length cDNA clones for the D4.2 and D4.4 forms of the receptor were made by *in vitro* recombination between partial cDNA clones of these forms with the full-length cDNA clone of the D4.7 receptor variant. The clone pCD-D4.4 was created by substituting the 920-bp *PstI-EcoRI* 3' fragment of pCD-D4.7 with the 730-bp *PstI-EcoRI* fragment of the D4 cDNA isolated from human pituitary. In a similar fashion the clone pCD-D4.2 was constructed by exchange of this 3' *PstI-EcoRI* fragment of pCD-D4.7 with a 630-bp *PstI-EcoRI* fragment of the D4.2 cDNA clone isolated from the neuroepithelioma SK-N-MC.

Transient expression in COS-7 cells was achieved as follows. Cells harvested and washed in phosphate buffered saline (PBS). 5×10^7 cells were resuspended in 1 ml PBS with 100 $\mu\text{g/ml}$ plasmid DNA (purified by caesium chloride gradient centrifugation) and incubated for 10 min on ice. Next, 400 μl aliquots of the cell suspension were subjected to an electric field of 0.65 kV/cm, 4.1 ms pulse duration using a BTX 600 Electro Cell Manipulator (Biotechnologies & Experimental Research, Inc., San Diego, CA). After the electric pulse, the cells were incubated for another 10 min on ice and then seeded in Modified Eagle's Medium supplemented with 10% fetal calf serum. The next day the medium was renewed. Three days after electroporation the cells were harvested and stored at -80°C until use in receptor binding studies as described herein.

Expression of each of the cloned variant D4 receptor constructs was demonstrated by Northern blot analysis as described in Example 1. Blots were hybridized with the 700 bp *EcoRI-XhoI* fragment of the D4 cDNA isolated from

the neuroepithelioma SK-N-MC (Example 2). The results of these experiments are shown in Figure 8. Transient expression of the three forms in COS-7 cells as characterized in these experiments demonstrated the expected size and size differences between the three forms, indicating that none of the expressed D4 receptor RNAs are further processed or produced from one another by RNA splicing events. Furthermore, the two bands observed for the D4.2 and D4.4 clones represent the consequence of the use of either the endogenous D4 receptor polyadenylation signal or the SV40 (vector-derived) polyadenylation signal). These observations indicate that in the transient expression system the expression of the three different clones would result in the formation of three structurally different receptors.

EXAMPLE 10

Analysis of Dopamine and Dopamine-Antagonist Binding of Variant D4 Dopamine Receptors

Pharmacological analysis of dopamine agonist and antagonist binding was performed as described in Example 6. The results of these experiments are shown in Figure 9. Panels (a) illustrate Scatchard analysis of the saturation isotherms for [3 H]spiperone binding to membranes prepared from COS-7 cells transiently transfected with pCD-D4.2 (D4.2), pCD-D4.4 (D4.4) and pCD-D4.7 (D4.7). Panels (b) show clozapine competition of [3 H]spiperone binding for the three allelic forms of the D4 receptor in the presence (+Na $^+$) and absence (-Na $^+$) of sodium chloride.

Pharmacological analysis demonstrated that all three variants displayed saturable [3 H]spiperone binding (300-1000 fmol mg $^{-1}$) with similar dissociation constants in the absence of sodium chloride (K_d = 40-50 pM; Figure 4a). However, in the presence of 120 mM sodium chloride, the dissociation constants increased approximately two- to three-fold for D4.2 and D4.4 but not for D4.7.

Clozapine competition of [3 H]spiperone binding revealed that D4.2 and D4.4 had lower dissociation constants for clozapine in the absence of sodium chloride (K_i = 3nM without sodium chloride; K_i = 23nM with sodium chloride). D4.7 had a dissociation constant of approximately 15 nM for clozapine which did

not exhibit sodium chloride sensitivity ($K_i = 12\text{nM}$ without sodium chloride; $K_i = 18\text{nM}$ with sodium chloride; shown in Figure 4b). This sodium chloride-mediated effect for clozapine on the D4 variants was not modulated by guanine nucleotides.

5 Agonists and antagonists (dopamine, bromocriptine, raclopride and clozapine) inhibited [^3H]spiperone binding (in the presence of sodium chloride) to these different D4 receptor variants in a concentration-dependent manner with similar dissociations constants. Furthermore, all three variants exhibited a
10 guanine nucleotide-sensitive high-affinity form of the receptor upon competition with dopamine, suggesting that all these variants can functionally couple to G-proteins. Thus, we have defined a novel, polymorphic dopamine receptor which we term D4.

15 It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: A Novel Human Dopamine Receptor and Uses

(iii) NUMBER OF SEQUENCES: 22

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: PCT/US93/_____

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 388 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..103

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 104..388

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..388

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Van Tol, Hubert H.M.
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Niznik, Hyman B.
Jovanovic, Vera
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- (D) VOLUME: 358

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Civelli, Olivier

(B) TITLE: Cloning of the gene for a human dopamine D4
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(C) JOURNAL: Nature

(D) VOLUME: 350

(F) PAGES: 610-614

(G) DATE: 18 April-1991

(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 388

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Met Gly Asn Arg	
1	
AGC ACC GCG GAC GCG GAC GGG CTG CTG GCT GGG CGC GGG CGG GCC GCG	163
Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg Gly Arg Ala Ala	
5 10 15 20	
GGG GCA TCT GCG GGG GCA TCT GCG GGG CTG GCT GGG CAG GGC GCG GCG	211
Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly Gln Gly Ala Ala	
25 30 35	
GCG CTG GTG GGG GGC GTG CTG CTC ATC GGC GCG GTG CTC GCG GGG AAC	259
Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val Leu Ala Gly Asn	
40 45 50	
TCG CTC GTG TGC GTG AGC GTG GCC ACC GAG CGC GCC CTG CAG ACG CCC	307
Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala Leu Gln Thr Pro	
55 60 65	
ACC AAC TCC TTC ATC GTG AGC CTG GCG GCC GCC GAC CTC CTC CTC GCT	355
Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp Leu Leu Leu Ala	
70 75 80	
CTC CTG GTG CTG CCG CTC TTC GTC TAC TCC GAG	388
Leu Leu Val Leu Pro Leu Phe Val Tyr Ser Glu	
85 90 95	

(2) INFORMATION FOR SEQ ID NO:2:

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(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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 20 25 30
 Gln Gly Ala Ala Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val
 35 40 45
 Leu Ala Gly Asn Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala
 50 55 60
 Leu Gln Thr Pro Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp
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(2) INFORMATION FOR SEQ ID NO:3:

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- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1..20
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /partial
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 estimated to be 2.0 kilobases in length"

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- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

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- (B) LOCATION: 1..20
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /partial
 /cons_splice= (5'site: NO, 3'site: YES)
 /evidence= EXPERIMENTAL

/label= IntronI
 /note= "This is the 3' sequence of a intron
 estimated to be 2.0 kilobases in length."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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20

(2) INFORMATION FOR SEQ ID NO:5:

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- (A) LENGTH: 113 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..113

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..113

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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1 5 10 15	
ATG GCC ATG GAC GTC ATG CTG TGC ACC GCC TCC ATC TTC AAC CTG TGC	96
Met Ala Met Asp Val Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys	
20 25 30	
GCC ATC AGC GTG GAC AG	113
Ala Ile Ser Val Asp	
35	

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Ala Ile Ser Val Asp	
35	

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1..102
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/label= IntronII

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GTGCGCCGCC CTCCCCGCC GCGCCCCGGC GCCCCCGCGC CCGCCCCGCC GCCCTCACCG      60
CGGCCTGTGC GCTGTCCGGC GCCCCCTCGG CGTCCCCGCG AG                        102
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 563 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..563
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard name= "Alternate Exon 3: D4.2"
/note= "This sequence represent the sequence of
the third exon of allele D4.2 of the human D4
dopamine receptor gene"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 257..262
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /function= "Polymorphic PstI site"
/evidence= EXPERIMENTAL
/label= PstI
/note= "This feature is the site of one of the
restriction enzymes whereby digestion of genomic
DNA produces a RFLP "

(ix) FEATURE:

- (A) NAME/KEY: repeat_region
- (B) LOCATION: 346..442
- (D) OTHER INFORMATION: /rpt_type= "tandem"
/rpt_unit= 348 .. 396
/note= "This sequence represents ne of 7 known
alleles of human D4 dopamine receptor gene
encoding a 16 amino acid sequence repeated twice

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 2..563

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

G TTC GTG GCC GTG GCC GTG CCG CTG CGC TAC AAC CGG CAG GGT GGG Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly 1 5 10 15	46
AGC CGC CGG CAG CTG CTG CTC ATC GGC GCC ACG TGG CTG CTG TCC GCG Ser Arg Arg Gln Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala 20 25 30	94
GCG GTG GCG GCG CCC GTA CTG TGC GGC CTC AAC GAC GTG CGC GGC CGC Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg 35 40 45	142
GAC CCC GCC GTG TGC CGC CTG GAG GAC CGC GAC TAC GTG GTC TAC TCG Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser 50 55 60	190
TCC GTG TGC TCC TTC TTC CTA CCC TGC CCG CTC ATG CTG CTG CTG TAC Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr 65 70 75	238
TGG GCC ACG TTC CGC GGC CTG CAG CGC TGG GAG GTG GCA CGT CGC GCC Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala 80 85 90 95	286
AAG CTG CAC GGC CGC GCG CCC CGC CGA CCC AGC GGC CCT GGC CCG CCT Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro 100 105 110	334
TCC CCC ACG CCA CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro 115 120 125	382
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC TCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser 130 135 140	430
AAC TGT GCT CCC CCC GAC GCC GTC AGA GCC GCC GCG CTC CCA CCC CAG Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln 145 150 155	478
ACT CCA CCG CAG ACC CGC AGG AGG CCG CGT GCC AAG ATC ACC GGC CGG Thr Pro Pro Gln Thr Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg 160 165 170 175	526
GAG CGC AAG GCC ATG AGG GTC CTG CCG GTG GTG GTC G Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val 180 185	563

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 187 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly Ser
 1 5 10 15
 Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala Ala
 20 25 30
 Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg Asp
 35 40 45
 Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser Ser
 50 55 60
 Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr Trp
 65 70 75 80
 Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala Lys
 85 90 95
 Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro Ser
 100 105 110
 Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro Asp
 115 120 125
 Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser Asn
 130 135 140
 Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln Thr
 145 150 155 160
 Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg Glu
 165 170 175
 Arg Lys Ala Met Arg Val Leu Pro Val Val Val
 180 185

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 659 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..659
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
 /standard name= "Alternate Exon 3: D4.4"
 /note= "This sequence represents the third exon of
 allele D4.4 of the human D4 dopamine receptor
 gene"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 257..262
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /function= "PstI site"
 /evidence= EXPERIMENTAL

/standard_name= "PstI site"
 /label= PstI
 /note= "This sequence represents a polymorphic
 PstI site whereby digestion of human genomic DNA
 produces a RFLP "

(ix) FEATURE:

- (A) NAME/KEY: repeat region
 (B) LOCATION: 346..538
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /rpt_type= "tandem"
 /evidence= EXPERIMENTAL
 /rpt_unit= 348 .. 396
 /note= "This repeat is present in 7 known alleles
 of the human D4 dopamine receptor gene and encodes
 a 16 amino acid sequence repeated 4 times in the

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 2..659

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

G TTC GTG GCC GTG GCC GTG CCG CTG CGC TAC AAC CGG CAG GGT GGG	46
Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly	
1 5 10 15	
AGC CGC CGG CAG CTG CTG CTC ATC GGC GCC ACG TGG CTG CTG TCC GCG	94
Ser Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala	
20 25 30	
GCG GTG GCG GCG CCC GTA CTG TGC GGC CTC AAC GAC GTG CGC GGC CGC	142
Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg	
35 40 45	
GAC CCC GCC GTG TGC CGC CTG GAG GAC CGC GAC TAC GTG GTC TAC TCG	190
Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser	
50 55 60	
TCC GTG TGC TCC TTC TTC CTA CCC TGC CCG CTC ATG CTG CTG CTG TAC	238
Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr	
65 70 75	
TGG GCC ACG TTC CGC GGC CTG CAG CGC TGG GAG GTG GCA CGT CGC GCC	286
Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala	
80 85 90 95	
AAG CTG CAC GGC CGC GCG CCC CGC CGA CCC AGC GGC CCT GGC CCG CCT	334
Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro	
100 105 110	
TCC CCC ACG CCA CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC	382
Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro	
115 120 125	
GAC TGT GCG CCC CCC GCG CCC GGC CTT CCC CGG GGT CCC TGC GGC CCC	430
Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro	
130 135 140	
GAC TGT GCG CCC GCC GCG CCC AGC CTC CCC CAG GAC CCC TGC GGC CCC	478
Asp Cys Ala Pro Ala Ala Pro Ser Leu Pro Gln Asp Pro Cys Gly Pro	
145 150 155	

GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC TCC	526
Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser	
160 165 170 175	
AAC TGT GCT CCC CCC GAC GCC GTC AGA GCC GCC GCG CTC CCA CCC CAG	574
Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln	
180 185 190	
ACT CCA CCG CAG ACC CGC AGG AGG CCG CGT GCC AAG ATC ACC GGC CCG	622
Thr Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg	
195 200 205	
GAG CGC AAG GCC ATG AGG GTC CTG CCG GTG GTG GTC G	659
Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val	
210 215	

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 219 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly Ser	
1 5 10 15	
Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala Ala	
20 25 30	
Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg Asp	
35 40 45	
Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser Ser	
50 55 60	
Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr Trp	
65 70 75 80	
Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala Lys	
85 90 95	
Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro Ser	
100 105 110	
Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro Asp	
115 120 125	
Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro Asp	
130 135 140	
Cys Ala Pro Ala Ala Pro Ser Leu Pro Gln Asp Pro Cys Gly Pro Asp	
145 150 155 160	
Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser Asn	
165 170 175	
Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln Thr	
180 185 190	
Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg Glu	
195 200 205	

Arg Lys Ala Met Arg Val Leu Pro Val Val Val
 210 215

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 803 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..803
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
 /standard name= "Alternate Exon 3: D4.7"
 /note= "This sequence represents the third exon of allele D4.7 of the human D4 dopamine receptor gene"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 257..262
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /function= "PstI site"
 /evidence= EXPERIMENTAL
 /standard name= "PstI site"
 /label= PstI
 /note= "This sequence is a PstI site whereby digestion of human genomic DNA produces a RFLP"

(ix) FEATURE:

- (A) NAME/KEY: repeat_region
- (B) LOCATION: 346..682
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /rpt_type= "tandem"
 /evidence= EXPERIMENTAL
 /rpt_unit= 346 .. 394
 /note= "This sequence is a repeat found in 7 known alleles of the human D4 dopamine receptor gene encoding a 16 amino acid sequence repeated 7 times"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..803

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

G	TTC	GTG	GCC	GTG	GCC	GTG	CCG	CTG	CGC	TAC	AAC	CGG	CAG	GGT	GGG	46
Phe	Val	Ala	Val	Ala	Val	Pro	Leu	Arg	Tyr	Asn	Arg	Gln	Gly	Gly		
1				5					10					15		
AGC	CGC	CCG	CAG	CTG	CTG	CTC	ATC	GGC	GCC	ACG	TGG	CTG	CTG	TCC	GCG	94
Ser	Arg	Arg	Gln	Leu	Leu	Leu	Ile	Gly	Ala	Thr	Trp	Leu	Leu	Ser	Ala	
				20				25						30		
GCG	GTG	GCG	GCG	CCC	GTA	CTG	TGC	GGC	CTC	AAC	GAC	GTG	CGC	GGC	CGC	142
Ala	Val	Ala	Ala	Pro	Val	Leu	Cys	Gly	Leu	Asn	Asp	Val	Arg	Gly	Arg	
				35				40						45		

GAC CCC GCC GTG TGC CGC CTG GAG GAC CGC GAC TAC GTG GTC TAC TCG Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser	190
50 55 60	
TCC GTG TGC TCC TTC TTC CTA CCC TGC CCG CTC ATG CTG CTG CTG TAC Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr	238
65 70 75	
TGG GCC ACG TTC CGC GGC CTG CAG CGC TGG GAG GTG GCA CGT CGC GCC Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala	286
80 85 90 95	
AAG CTG CAC GGC CGC GCG CCC CGC CGA CCC AGC GGC CCT GGC CCG CCT Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro	334
100 105 110	
TCC CCC ACG CCA CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Pro Gln Asp Pro Cys Gly Pro	382
115 120 125	
GAC TGT GCG CCC CCC GCG CCC GGC CTT CCC CGG GGT CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro	430
130 135 140	
GAC TGT GCG CCC GCC GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC CCC Asp Cys Ala Pro Ala Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Pro	478
145 150 155	
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CAG GAC CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp Pro Cys Gly Pro	526
160 165 170 175	
GAC TGT GCG CCC CCC GCG CCC GGC CTT CCC CGG GGT CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro	574
180 185 190	
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CAG GAC CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp Pro Cys Gly Pro	622
195 200 205	
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC TCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser	670
210 215 220	
AAC TGT GCT CCC CCC GAC GCC GTC AGA GCC GCC GCG CTC CCA CCC CAG Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln	718
225 230 235	
ACT CCA CCG CAG ACC CGC AGG AGG CGG CGT GCC AAG ATC ACC GGC CGG Thr Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg	766
240 245 250 255	
GAG CGC AAG GCC ATG AGG GTC CTG CCG GTG GTG GTC G Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val	803
260 265	

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 267 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: prot in

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly Ser
 1 5 10 15
 Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala Ala
 20 25 30
 Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg Asp
 35 40 45
 Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser Ser
 50 55 60
 Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr Trp
 65 70 75 80
 Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala Lys
 85 90 95
 Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro Ser
 100 105 110
 Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro Asp
 115 120 125
 Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro Asp
 130 135 140
 Cys Ala Pro Ala Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Pro Asp
 145 150 155 160
 Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp Pro Cys Gly Pro Asp
 165 170 175
 Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro Asp
 180 185 190
 Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp Pro Cys Gly Pro Asp
 195 200 205
 Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser Asn
 210 215 220
 Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln Thr
 225 230 235 240
 Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg Glu
 245 250 255
 Arg Lys Ala Met Arg Val Leu Pro Val Val Val
 260 265

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (gen mic)

(ix) FEATURE:

(A) NAME/KEY: intron
(B) LOCATI N: 1..94

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGGGTTTCCT GTCCTGAGGG GCGGGGAGGA GAGGAGGGGG GGAGTACGAG GCCGGCTGGG 60
CGGGGGGCGC TAACGCGGCT CTCGGCGCCC CCAG 94

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 328 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 1..328

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 3..203

(ix) FEATURE:

(A) NAME/KEY: 3'UTR
(B) LOCATION: 204..328

(ix) FEATURE:

(A) NAME/KEY: polyA_site
(B) LOCATION: 304

(ix) FEATURE:

(A) NAME/KEY: misc feature
(B) LOCATION: 36..41
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /function= "HincII site"
/evidence= EXPERIMENTAL
/standard name= "HincII site"
/label= HincII
/note= "This sequence is a HincII site whereby
digestion of genomic DNA produces a RFLP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GG GCC TTC CTG CTG TGC TGG ACG CCC TTC TTC GTG GTG CAC ATC ACG 47
Ala Phe Leu Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr
1 5 10 15
CAG GCG CTG TGT CCT GCC TGC TCC GTG CCC CCG CGG CTG GTC AGC GCC 95
Gln Ala Leu Cys Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala
20 25 30
GTC ACC TGG CTG GGC TAC GTC AAC AGC GCC CTC ACC CCC GTC ATC TAC 143
Val Thr Trp Leu Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr
35 40 45

ACT TC TTC AAC GCC GAG TTC CGC AAC GTC TTC CGC AAG GCC CTG CGT 191
 Thr Val Phe Asn Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg
 50 55 60

GCC TGC TGC TGAGCCGGGC ACCCCCGGAC GCCCCCGGC CTGATGGCCA 240
 Ala Cys Cys
 65

GGCCTCAGGG ACCAAGGAGA TGGGGAGGGC GCTTTTGTAC GTTAATTAAA CAAATTCCTT 300
 CCCAAACTCA GCTGTGAAGG CTCCTGGG 328

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Phe Leu Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr Gln
 1 5 10 15

Ala Leu Cys Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala Val
 20 25 30

Thr Trp Leu Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr Thr
 35 40 45

Val Phe Asn Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg Ala
 50 55 60

Cys Cys
 65

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1370 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..103

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 1268..1370

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..1267

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGGGGGCGGG ACCAGGTCC GGCCGGGGCG TCCCCCGGG GAGGGACTCC CCGGCTTGCC	60
CCCCGGCGTT GTCCGCGGTG CTCAGCGCCC GCCCGGGCGC GCC ATG GGG AAC CGC	115
Met Gly Asn Arg	
1	
AGC ACC GCG GAC GCG GAC GGG CTG CTG GCT GGG CGC GGG CGG GCC GCG	163
Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg Gly Arg Ala Ala	
5 10 15 20	
GGG GCA TCT GCG GGG GCA TCT GCG GGG CTG GCT GGG CAG GGC GCG GCG	211
Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly Gln Gly Ala Ala	
25 30 35	
GCG CTG GTG GGG GGC GTG CTG CTC ATC GGC GCG GTG CTC GCG GGG AAC	259
Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val Leu Ala Gly Asn	
40 45 50	
TCG CTC GTG TGC GTG AGC GTG GCC ACC GAG CGC GCC CTG CAG ACG CCC	307
Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala Leu Gln Thr Pro	
55 60 65	
ACC AAC TCC TTC ATC GTG AGC CTG GCG GCC GCC GAC CTC CTC CTC GCT	355
Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp Leu Leu Leu Ala	
70 75 80	
CTC CTG GTG CTG CCG CTC TTC GTC TAC TCC GAG GTC CAG GGT GGC GCG	403
Leu Leu Val Leu Pro Leu Phe Val Tyr Ser Glu Val Gln Gly Gly Ala	
85 90 95 100	
TGG CTG CTG AGC CCC CGC CTG TGC GAC GCC CTC ATG GCC ATG GAC GTC	451
Trp Leu Leu Ser Pro Arg Leu Cys Asp Ala Leu Met Ala Met Asp Val	
105 110 115	
ATG CTG TGC ACC GCC TCC ATC TTC AAC CTG TGC GCC ATC AGC GTG GAC	499
Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys Ala Ile Ser Val Asp	
120 125 130	
AGG TTC GTG GCC GTG GCC GTG CCG CTG CGC TAC AAC CCG CAG GGT GGG	547
Arg Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly	
135 140 145	
AGC CGC CGG CAG CTG CTG CTC ATC GGC GCC ACG TGG CTG CTG TCC GCG	595
Ser Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala	
150 155 160	
GCG GTG GCG GCG CCC GTA CTG TGC GGC CTC AAC GAC GTG CGC GGC CGC	643
Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg	
165 170 175 180	
GAC CCC GCC GTG TGC CGC CTG GAG GAC CGC GAC TAC GTG GTC TAC TCG	691
Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser	
185 190 195	
TCC GTG TGC TCC TTC TTC CTA CCC TGC CCG CTC ATG CTG CTG CTG TAC	739
Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr	
200 205 210	
TGG GCC ACG TTC CGC GGC CTG CAG CGC TGG GAG GTG GCA CGT CGC GCC	787
Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala	
215 220 225	

AAG CTG CAC GGC CGC GCG CCC CGC CGA CCC AGC GGC CCT GGC CCG CCT 835
 Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro
 230 235 240
 TCC CCC ACG CCA CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC 883
 Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro
 245 250 255 260
 GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC TCC 931
 Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser
 265 270 275
 AAC TGT GCT CCC CCC GAC GCC GTC AGA GCC GCC GCG CTC CCA CCC CAG 979
 Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln
 280 285 290
 ACT CCA CCG CAG ACC CGC AGG AGG CCG CGT GCC AAG ATC ACC GGC CCG 1027
 Thr Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg
 295 300 305
 GAG CGC AAG GCC ATG AGG GTC CTG CCG GTG GTG GTC GGG GCC TTC CTG 1075
 Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val Gly Ala Phe Leu
 310 315 320
 CTG TGC TGG ACG CCC TTC TTC GTG GTG CAC ATC ACG CAG GCG CTG TGT 1123
 Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr Gln Ala Leu Cys
 325 330 335 340
 CCT GCC TGC TCC GTG CCC CCG CCG CTG GTC AGC GCC GTC ACC TGG CTG 1171
 Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala Val Thr Trp Leu
 345 350 355
 GGC TAC GTC AAC AGC GCC CTC ACC CCC GTC ATC TAC ACT GTC TTC AAC 1219
 Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr Thr Val Phe Asn
 360 365 370
 GCC GAG TTC CGC AAC GTC TTC CGC AAG GCC CTG CGT GCC TGC TGC TGAGCCGGGC 1274
 Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg Ala Cys Cys
 375 380 385
 ACCCCCCGAC GCCCCCGGC CTGATGGCCA GGCCTCAGGG ACCAAGGAGA TGGGGAGGGC 1334
 GCTTTTGTAC GTTAATTAAA CAAATTCCTT CCCAAA 1370

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 387 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Gly Asn Arg Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg
 1 5 10 15
 Gly Arg Ala Ala Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly
 20 25 30
 Gln Gly Ala Ala Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val
 35 40 45

Leu Ala Gly Asn Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala
 50 55 60
 Leu Gln Thr Pro Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp
 65 70 75 80
 Leu Leu Leu Ala Leu Val Leu Pro Leu Phe Val Tyr Ser Glu Val
 85 90 95
 Gln Gly Gly Ala Trp Leu Leu Ser Pro Arg Leu Cys Asp Ala Leu Met
 100 105 110
 Ala Met Asp Val Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys Ala
 115 120 125
 Ile Ser Val Asp Arg Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn
 130 135 140
 Arg Gln Gly Gly Ser Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp
 145 150 155 160
 Leu Leu Ser Ala Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp
 165 170 175
 Val Arg Gly Arg Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr
 180 185 190
 Val Val Tyr Ser Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met
 195 200 205
 Leu Leu Leu Tyr Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val
 210 215 220
 Ala Arg Arg Ala Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly
 225 230 235 240
 Pro Gly Pro Pro Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp
 245 250 255
 Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp
 260 265 270
 Pro Cys Gly Ser Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala
 275 280 285
 Leu Pro Pro Gln Thr Pro Pro Gln Thr Arg Arg Arg Arg Ala Lys
 290 295 300
 Ile Thr Gly Arg Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val
 305 310 315 320
 Gly Ala Phe Leu Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr
 325 330 335
 Gln Ala Leu Cys Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala
 340 345 350
 Val Thr Trp Leu Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr
 355 360 365
 Thr Val Phe Asn Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg
 370 375 380
 Ala Cys Cys
 385

(2) INFORMATION FOR SEQ ID NO:19:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1466 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
 (B) LOCATION: 1..103

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
 (B) LOCATION: 1364..1466

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 104..1363

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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CGGGGGCGGG ACCAGGGTCC GGCCGGGGCG TCCCCCGGG GAGGGACTCC CCGGCTTGCC      60
CCCCGGCGTT GTCCGCGGTG CTCAGCGCCC GCCCGGGCGC GCC ATG GGG AAC CGC      115
                                     Met Gly Asn Arg
                                     1

AGC ACC GCG GAC GCG GAC GGG CTG CTG GCT GGG CGC GGG CGG GCC GCG      163
Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg Gly Arg Ala Ala
  5              10              15              20

GGG GCA TCT GCG GGG GCA TCT GCG GGG CTG GCT GGG CAG GGC GCG GCG      211
Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly Gln Gly Ala Ala
                25              30              35

GCG CTG GTG GGG GGC GTG CTG CTC ATC GGC GCG GTG CTC GCG GGG AAC      259
Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val Leu Ala Gly Asn
                40              45              50

TCG CTC GTG TGC GTG AGC GTG GCC ACC GAG CGC GCC CTG CAG ACG CCC      307
Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala Leu Gln Thr Pro
                55              60              65

ACC AAC TCC TTC ATC GTG AGC CTG GCG GCC GCC GAC CTC CTC CTC GCT      355
Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp Leu Leu Leu Ala
                70              75              80

CTC CTG GTG CTG CCG CTC TTC GTC TAC TCC GAG GTC CAG GGT GGC GCG      403
Leu Leu Val Leu Pro Leu Phe Val Tyr Ser Glu Val Gln Gly Gly Ala
                85              90              95              100

TGG CTG CTG AGC CCC CGC CTG TGC GAC GCC CTC ATG GCC ATG GAC GTC      451
Trp Leu Leu Ser Pro Arg Leu Cys Asp Ala Leu Met Ala Met Asp Val
                105              110              115

ATG CTG TGC ACC GCC TCC ATC TTC AAC CTG TGC GCC ATC AGC GTG GAC      499
Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys Ala Ile Ser Val Asp
                120              125              130

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AGG	TTC	GTG	GCC	GTG	GCC	GTG	CCG	CTG	CGC	TAC	AAC	CGG	CAG	GGT	GGG	547
Arg	Phe	Val	Ala	Val	Ala	Val	Pro	Leu	Arg	Tyr	Asn	Arg	Gln	Gly	Gly	
		135					140					145				
AGC	CGC	CGG	CAG	CTG	CTG	CTC	ATC	GGC	GCC	ACG	TGG	CTG	CTG	TCC	GCG	595
Ser	Arg	Arg	Gln	Leu	Leu	Leu	Ile	Gly	Ala	Thr	Trp	Leu	Leu	Ser	Ala	
	150					155					160					
GCG	GTG	GCG	GCG	CCC	GTA	CTG	TGC	GGC	CTC	AAC	GAC	GTG	CGC	GGC	CGC	643
Ala	Val	Ala	Ala	Pro	Val	Leu	Cys	Gly	Leu	Asn	Asp	Val	Arg	Gly	Arg	
	165				170				175						180	
GAC	CCC	GCC	GTG	TGC	CGC	CTG	GAG	GAC	CGC	GAC	TAC	GTG	GTC	TAC	TCG	691
Asp	Pro	Ala	Val	Cys	Arg	Leu	Glu	Asp	Arg	Asp	Tyr	Val	Val	Tyr	Ser	
				185					190					195		
TCC	GTG	TGC	TCC	TTC	TTC	CTA	CCC	TGC	CCG	CTC	ATG	CTG	CTG	CTG	TAC	739
Ser	Val	Cys	Ser	Phe	Phe	Leu	Pro	Cys	Pro	Leu	Met	Leu	Leu	Leu	Tyr	
			200					205					210			
TGG	GCC	ACG	TTC	CGC	GGC	CTG	CAG	CGC	TGG	GAG	GTG	GCA	CGT	CGC	GCC	787
Trp	Ala	Thr	Phe	Arg	Gly	Leu	Gln	Arg	Trp	Glu	Val	Ala	Arg	Arg	Ala	
		215					220					225				
AAG	CTG	CAC	GGC	CGC	GCG	CCC	CGC	CGA	CCC	AGC	GGC	CCT	GGC	CCG	CCT	835
Lys	Leu	His	Gly	Arg	Ala	Pro	Arg	Arg	Pro	Ser	Gly	Pro	Gly	Pro	Pro	
	230					235					240					
TCC	CCC	ACG	CCA	CCC	GCG	CCC	CGC	CTC	CCC	CAG	GAC	CCC	TGC	GGC	CCC	883
Ser	Pro	Thr	Pro	Pro	Ala	Pro	Arg	Leu	Pro	Gln	Asp	Pro	Cys	Gly	Pro	
	245				250					255					260	
GAC	TGT	GCG	CCC	CCC	GCG	CCC	GGC	CTT	CCC	CGG	GGT	CCC	TGC	GGC	CCC	931
Asp	Cys	Ala	Pro	Pro	Ala	Pro	Gly	Leu	Pro	Arg	Gly	Pro	Cys	Gly	Pro	
				265					270					275		
GAC	TGT	GCG	CCC	GCC	GCG	CCC	AGC	CTC	CCC	CAG	GAC	CCC	TGC	GGC	CCC	979
Asp	Cys	Ala	Pro	Ala	Ala	Pro	Ser	Leu	Pro	Gln	Asp	Pro	Cys	Gly	Pro	
			280					285					290			
GAC	TGT	GCG	CCC	CCC	GCG	CCC	GGC	CTC	CCC	CCG	GAC	CCC	TGC	GGC	TCC	1027
Asp	Cys	Ala	Pro	Pro	Ala	Pro	Gly	Leu	Pro	Pro	Asp	Pro	Cys	Gly	Ser	
		295					300					305				
AAC	TGT	GCT	CCC	CCC	GAC	GCC	GTC	AGA	GCC	GCC	GCG	CTC	CCA	CCC	CAG	1075
Asn	Cys	Ala	Pro	Pro	Asp	Ala	Val	Arg	Ala	Ala	Ala	Leu	Pro	Pro	Gln	
	310					315					320					
ACT	CCA	CCG	CAG	ACC	CGC	AGG	AGG	CGG	CGT	GCC	AAG	ATC	ACC	GGC	CGG	1123
Thr	Pro	Pro	Gln	Thr	Arg	Arg	Arg	Arg	Arg	Ala	Lys	Ile	Thr	Gly	Arg	
					330				335						340	
GAG	CGC	AAG	GCC	ATG	AGG	GTC	CTG	CCG	GTG	GTG	GTC	GGG	GCC	TTC	CTG	1171
Glu	Arg	Lys	Ala	Met	Arg	Val	Leu	Pro	Val	Val	Val	Gly	Ala	Phe	Leu	
				345					350					355		
CTG	TGC	TGG	ACG	CCC	TTC	TTC	GTG	GTG	CAC	ATC	ACG	CAG	GCG	CTG	TGT	1219
Leu	Cys	Trp	Thr	Pro	Phe	Phe	Val	Val	His	Ile	Thr	Gln	Ala	Leu	Cys	
			360				365						370			
CCT	GCC	TGC	TCC	GTG	CCC	CCG	CGG	CTG	GTC	AGC	GCC	GTC	ACC	TGG	CTG	1267
Pro	Ala	Cys	Ser	Val	Pro	Pro	Arg	Leu	Val	Ser	Ala	Val	Thr	Trp	Leu	
		375					380					385				

GGC TAC GTC AAC AGC GCC CTC ACC CCC GTC ATC TAC ACT GTC TTC AAC 1315
 Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr Thr Val Phe Asn
 390 395 400

GCC GAG TTC CGC AAC GTC TTC CGC AAG GCC CTG CGT GCC TGC TGC TGAGCCGGGC 1370
 Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg Ala Cys Cys
 405 410 415 420

ACCCCCGGAC GCCCCCGGC CTGATGGCCA GGCCTCAGGG ACCAAGGAGA TGGGGAGGGC 1430
 GCTTTTGTAC GTTAATTAAA CAAATTCCTT CCCAAA 1466

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 419 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Gly Asn Arg Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg
 1 5 10 15

Gly Arg Ala Ala Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly
 20 25 30

Gln Gly Ala Ala Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val
 35 40 45

Leu Ala Gly Asn Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala
 50 55 60

Leu Gln Thr Pro Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp
 65 70 75 80

Leu Leu Leu Ala Leu Leu Val Leu Pro Leu Phe Val Tyr Ser Glu Val
 85 90 95

Gln Gly Gly Ala Trp Leu Leu Ser Pro Arg Leu Cys Asp Ala Leu Met
 100 105 110

Ala Met Asp Val Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys Ala
 115 120 125

Ile Ser Val Asp Arg Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn
 130 135 140

Arg Gln Gly Gly Ser Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp
 145 150 155 160

Leu Leu Ser Ala Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp
 165 170 175

Val Arg Gly Arg Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr
 180 185 190

Val Val Tyr Ser Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met
 195 200 205

Leu Leu Leu Tyr Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val
 210 215 220

Ala Arg Arg Ala Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly
 225 230 235 240
 Pro Gly Pr Pro Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp
 245 250 255
 Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly
 260 265 270
 Pro Cys Gly Pro Asp Cys Ala Pro Ala Ala Pro Ser Leu Pro Gln Asp
 275 280 285
 Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp
 290 295 300
 Pro Cys Gly Ser Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala
 305 310 315 320
 Leu Pro Pro Gln Thr Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys
 325 330 335
 Ile Thr Gly Arg Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val
 340 345 350
 Gly Ala Phe Leu Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr
 355 360 365
 Gln Ala Leu Cys Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala
 370 375 380
 Val Thr Trp Leu Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr
 385 390 395 400
 Thr Val Phe Asn Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg
 405 410 415
 Ala Cys Cys

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1610 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..103

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 1508..1610

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..1507

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGGGGGCGGG ACCAGGGTCC GGCCGGGGCG TGGCCCCGGG GAGGGACTCC CCGGCTTGCC	60
CCCCGGCGTT GTCCGCGGTG CTCAGCGCCC GCGGGGCGC GCC ATG GGG AAC CGC	115
Met Gly Asn Arg	
1	
AGC ACC GCG GAC GCG GAC GGG CTG CTG GCT GGG CGC GGG CGG GCC GCG	163
Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg Gly Arg Ala Ala	
5 10 15 20	
GGG GCA TCT GCG GGG GCA TCT GCG GGG CTG GCT GGG CAG GGC GCG GCG	211
Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly Gln Gly Ala Ala	
25 30 35	
GCG CTG GTG GGG GGC GTG CTG CTC ATC GGC GCG GTG CTC GCG GGG AAC	259
Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val Leu Ala Gly Asn	
40 45 50	
TCG CTC GTG TGC GTG AGC GTG GCC ACC GAG CGC GCC CTG CAG ACG CCC	307
Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala Leu Gln Thr Pro	
55 60 65	
ACC AAC TCC TTC ATC GTG AGC CTG GCG GCC GCC GAC CTC CTC CTC GCT	355
Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp Leu Leu Leu Ala	
70 75 80	
CTC CTG GTG CTG CCG CTC TTC GTC TAC TCC GAG GTC CAG GGT GGC GCG	403
Leu Leu Val Leu Pro Leu Phe Val Tyr Ser Glu Val Gln Gly Gly Ala	
85 90 95 100	
TGG CTG CTG AGC CCC CGC CTG TGC GAC GCC CTC ATG GCC ATG GAC GTC	451
Trp Leu Leu Ser Pro Arg Leu Cys Asp Ala Leu Met Ala Met Asp Val	
105 110 115	
ATG CTG TGC ACC GCC TCC ATC TTC AAC CTG TGC GCC ATC AGC GTG GAC	499
Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys Ala Ile Ser Val Asp	
120 125 130	
AGG TTC GTG GCC GTG GCC GTG CCG CTG CGC TAC AAC CGG CAG GGT GGG	547
Arg Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn Arg Gln Gly Gly	
135 140 145	
AGC CGC CGG CAG CTG CTG CTC ATC GGC GCC ACG TGG CTG CTG TCC GCG	595
Ser Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp Leu Leu Ser Ala	
150 155 160	
GCG GTG GCG GCG CCC GTA CTG TGC GGC CTC AAC GAC GTG CGC GGC CGC	643
Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp Val Arg Gly Arg	
165 170 175 180	
GAC CCC GCC GTG TGC CGC CTG GAG GAC CGC GAC TAC GTG GTC TAC TCG	691
Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr Val Val Tyr Ser	
185 190 195	
TCC GTG TGC TCC TTC TTC CTA CCC TGC CCG CTC ATG CTG CTG CTG TAC	739
Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met Leu Leu Leu Tyr	
200 205 210	
TGG GCC ACG TTC CGC GGC CTG CAG CGC TGG GAG GTG GCA CGT CGC GCC	787
Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val Ala Arg Arg Ala	
215 220 225	

AAG CTG CAC GGC CGC GCG CCC CGC CGA CCC AGC GGC CCT GGC CCG CCT Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly Pro Gly Pro Pro 230 235 240	835
TCC CCC ACG CCA CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp Pro Cys Gly Pro 245 250 255 260	883
GAC TGT GCG CCC CCC GCG CCC GGC CTT CCC CGG GGT CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro 265 270 275	931
GAC TGT GCG CCC GCG GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC CCC Asp Cys Ala Pro Ala Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Pro 280 285 290	979
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CAG GAC CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp Pro Cys Gly Pro 295 300 305	1027
GAC TGT GCG CCC CCC GCG CCC GGC CTT CCC CGG GGT CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly Pro Cys Gly Pro 310 315 320	1075
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CAG GAC CCC TGC GGC CCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp Pro Cys Gly Pro 325 330 335 340	1123
GAC TGT GCG CCC CCC GCG CCC GGC CTC CCC CCG GAC CCC TGC GGC TCC Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp Pro Cys Gly Ser 345 350 355	1171
AAC TGT GCT CCC CCC GAC GCC GTC AGA GCC GCC GCG CTC CCA CCC CAG Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala Leu Pro Pro Gln 360 365 370	1219
ACT CCA CCG CAG ACC CGC AGG AGG CGG CGT GCC AAG ATC ACC GGC CGG Thr Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys Ile Thr Gly Arg 375 380 385	1267
GAG CGC AAG GCC ATG AGG GTC CTG CCG GTG GTG GTC GGG GCC TTC CTG Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val Gly Ala Phe Leu 390 395 400	1315
CTG TGC TGG ACG CCC TTC TTC GTG GTG CAC ATC ACG CAG GCG CTG TGT Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr Gln Ala Leu Cys 405 410 415 420	1363
CCT GCC TGC TCC GTG CCC CCG CGG CTG GTC AGC GCC GTC ACC TGG CTG Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala Val Thr Trp Leu 425 430 435	1411
GGC TAC GTC AAC AGC GCC CTC ACC CCC GTC ATC TAC ACT GTC TTC AAC Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr Thr Val Phe Asn 440 445 450	1459
GCC GAG TTC CGC AAC GTC TTC CGC AAG GCC CTG CGT GCC TGC TGC TGAGCCGGGC Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg Ala Cys Cys 455 460 465	1514
ACCCCCGGAC GCCCCCCGGC CTGATGGCCA GGCCTCAGGG ACCAAGGAGA TGGGGAGGGC	1574
GCTTTTGTAC GTTAATTAAA CAAATTCCTT CCCAAA	1610

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 467 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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Met Gly Asn Arg Ser Thr Ala Asp Ala Asp Gly Leu Leu Ala Gly Arg
 1           5           10           15
Gly Arg Ala Ala Gly Ala Ser Ala Gly Ala Ser Ala Gly Leu Ala Gly
          20           25           30
Gln Gly Ala Ala Ala Leu Val Gly Gly Val Leu Leu Ile Gly Ala Val
          35           40           45
Leu Ala Gly Asn Ser Leu Val Cys Val Ser Val Ala Thr Glu Arg Ala
          50           55           60
Leu Gln Thr Pro Thr Asn Ser Phe Ile Val Ser Leu Ala Ala Ala Asp
          65           70           75           80
Leu Leu Leu Ala Leu Leu Val Leu Pro Leu Phe Val Tyr Ser Glu Val
          85           90           95
Gln Gly Gly Ala Trp Leu Leu Ser Pro Arg Leu Cys Asp Ala Leu Met
          100          105          110
Ala Met Asp Val Met Leu Cys Thr Ala Ser Ile Phe Asn Leu Cys Ala
          115          120          125
Ile Ser Val Asp Arg Phe Val Ala Val Ala Val Pro Leu Arg Tyr Asn
          130          135          140
Arg Gln Gly Gly Ser Arg Arg Gln Leu Leu Leu Ile Gly Ala Thr Trp
          145          150          155          160
Leu Leu Ser Ala Ala Val Ala Ala Pro Val Leu Cys Gly Leu Asn Asp
          165          170          175
Val Arg Gly Arg Asp Pro Ala Val Cys Arg Leu Glu Asp Arg Asp Tyr
          180          185          190
Val Val Tyr Ser Ser Val Cys Ser Phe Phe Leu Pro Cys Pro Leu Met
          195          200          205
Leu Leu Leu Tyr Trp Ala Thr Phe Arg Gly Leu Gln Arg Trp Glu Val
          210          215          220
Ala Arg Arg Ala Lys Leu His Gly Arg Ala Pro Arg Arg Pro Ser Gly
          225          230          235          240
Pro Gly Pro Pro Ser Pro Thr Pro Pro Ala Pro Arg Leu Pro Gln Asp
          245          250          255
Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly
          260          265          270
Pro Cys Gly Pro Asp Cys Ala Pro Ala Ala Pro Gly Leu Pro Pro Asp
          275          280          285

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Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp
 290 295 300
 Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Arg Gly
 305 310 315 320
 Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Gln Asp
 325 330 335
 Pro Cys Gly Pro Asp Cys Ala Pro Pro Ala Pro Gly Leu Pro Pro Asp
 340 345 350
 Pro Cys Gly Ser Asn Cys Ala Pro Pro Asp Ala Val Arg Ala Ala Ala
 355 360 365
 Leu Pro Pro Gln Thr Pro Pro Gln Thr Arg Arg Arg Arg Arg Ala Lys
 370 375 380
 Ile Thr Gly Arg Glu Arg Lys Ala Met Arg Val Leu Pro Val Val Val
 385 390 395 400
 Gly Ala Phe Leu Leu Cys Trp Thr Pro Phe Phe Val Val His Ile Thr
 405 410 415
 Gln Ala Leu Cys Pro Ala Cys Ser Val Pro Pro Arg Leu Val Ser Ala
 420 425 430
 Val Thr Trp Leu Gly Tyr Val Asn Ser Ala Leu Thr Pro Val Ile Tyr
 435 440 445
 Thr Val Phe Asn Ala Glu Phe Arg Asn Val Phe Arg Lys Ala Leu Arg
 450 455 460
 Ala Cys Cys
 465

WHAT WE CLAIM IS:

1. A DNA sequence comprising a nucleotide sequence encoding a mammalian dopamine receptor, wherein the mammalian dopamine receptor has the drug dissociation properties of the human dopamine receptor D4.

5 2. The DNA sequence of Claim 1 wherein the mammalian dopamine receptor encoded is the human D4 dopamine receptor.

3. The DNA sequence of Claim 1 wherein the mammalian dopamine receptor encoded therein has the drug dissociation properties described in Table 1.

10 4. The DNA sequence of Claim 1 wherein the mammalian dopamine receptor encoded therein has a high affinity for the drug clozapine.

5. The DNA sequence of Claim 1 comprising a repeated DNA sequence that is substantially homologous to the sequence:

5'-A CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC GAC
TGT GCG CC-3'

15 6. The DNA sequence of Claim 5 comprising from about 2 to about 8 copies of the repeated DNA sequence.

7. The DNA sequence of Claim 5 having a sequence substantially homologous to allele D4.2 of the human D4 dopamine receptor gene [SEQ ID No.: 17].

20 8. The DNA sequence of Claim 5 having a sequence substantially homologous to allele D4.4 of the human D4 dopamine receptor gene [SEQ ID No.: 19].

25 9. The DNA sequence of Claim 5 having a sequence substantially homologous to allele D4.7 of the human D4 dopamine receptor gene [SEQ ID No.: 21].

10. A homogeneous composition of a 41 kilodalton human dopamine receptor D4 or derivative thereof, wherein the amino acid sequence of the dopamine receptor or derivative thereof is substantially homologous to the sequence in Figure 3.

30 11. The homogeneous composition of Claim 10 wherein the amino acid sequence of the dopamine receptor or derivative thereof comprises a repeated

amino acid sequence that is substantially homologous to the sequence:

(P/A)AP(R/G)LP(Q/R/P)(D/G)PCG(P/S)(D/N)CAP

12. The amino acid sequence of Claim 11 comprising from about 2 to about 8 copies of the repeated amino acid sequence.

5 13. The amino acid sequence of Claim 11 having a sequence substantially homologous to the amino acid sequence encoded by allele D4.2 of the human D4 dopamine receptor gene [SEQ ID No.: 18].

14. The amino acid sequence of Claim 11 having a sequence substantially homologous to the amino acid sequence encoded by allele D4.4 of the
10 human D4 dopamine receptor gene [SEQ ID No.: 20].

15. The amino acid sequence of Claim 11 having a sequence substantially homologous to the amino acid sequence encoded by allele D4.7 of the human D4 dopamine receptor gene [SEQ ID No.: 22].

16. A recombinant DNA construct comprising a nucleotide sequence
15 encoding the human dopamine receptor D4.

17. A recombinant expression construct comprising the DNA sequence of Claim 2, wherein the construct is capable of expressing the human dopamine receptor D4 in a transformed eukaryotic cell culture.

18. The recombinant expression vector of Claim 17 wherein the DNA
20 sequence comprises a repeated DNA sequence that is substantially homologous to the sequence:

5'-A CCC GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC GAC
TGT GCG CC-3'

19. The DNA sequence of Claim 18 comprising from about 2 to about
25 8 copies of the repeated DNA sequence.

20. The DNA sequence of Claim 18 having a sequence substantially homologous to allele D4.2 of the human D4 dopamine receptor gene [SEQ ID No.: 17].

21. The DNA sequence of Claim 18 having a sequence substantially
30 homologous to allele D4.4 of the human D4 dopamine receptor gene [SEQ ID No.: 19].

22. The DNA sequence of Claim 18 having a sequence substantially homologous to allele D4.7 of the human D4 dopamine receptor gene [SEQ ID No.: 21].

5 23. A eukaryotic cell culture transformed with the recombinant expression construct of Claim 17, wherein the transformed eukaryotic cell culture is capable of expressing the human dopamine receptor D4.

24. A method of screening a compound as an inhibitor of dopamine binding to the human dopamine receptor D4, the method comprising the following steps:

- 10 (a) transforming a eukaryotic cell culture with an expression vector as in Claim 17 capable of expressing the human dopamine receptor D4 in a eukaryotic cell; and
- (b) assaying for ability of the compound to inhibit the binding of a detectable dopamine analog.

15 25. A method of screening a compound for anti-psychotic activity, the method comprising the following steps:

- (a) transforming a eukaryotic cell culture with an expression vector as in Claim 17 capable of expressing the human dopamine receptor D4 in a eukaryotic cell;
- 20 (b) assaying for ability of the compound to inhibit the binding of a detectable dopamine analog; and
- (c) testing those drugs for anti-psychotic activity based on their affinity for the D4 dopamine receptor.

25 26. A method of quantitatively detecting a compound as an inhibitor of dopamine binding to the human dopamine receptor D4, the method comprising the following steps:

- (a) transforming a eukaryotic cell culture with an expression vector as in Claim 17 capable of expressing the human dopamine receptor D4 in a eukaryotic cell; and
- 30 (b) assaying for amount of a compound by measuring the extent of inhibition of binding of a detectable dopamine analog.

27. The method of Claim 26 wherein the compound to be tested is present in a human.

28. The method of Claim 26 wherein the compound is present in human blood.

5 29. The method of Claim 26 wherein the compound is present in human cerebrospinal fluid.

30. The method of Claim 26 wherein the compound is present in human brain.

31. The method of Claim 26 wherein the compound is unknown.

10 32. A method for detecting a restriction fragment length polymorphism in a gene encoding a D4 dopamine receptor in a human comprising the following steps:

- (a) isolating a sufficient quantity of DNA from the human;
- (b) digesting the DNA with a first restriction enzyme that is *Pst*I and a second restriction enzyme that is *Hinc*II to produce a
15 multiplicity of fragments of digested DNA;
- (c) analyzing the fragments of digested DNA by hybridization with a probe comprising the nucleic acid sequence of Claim 2; and
- 20 (d) detecting a pattern of the hybridized fragments of the human dopamine receptor gene.

33. A method for screening a population of humans to determine the frequency of restriction fragment length polymorphism of a gene encoding a D4 dopamine receptor comprising the following steps:

- 25 (a) detecting a pattern of DNA fragments of the gene encoding a dopamine receptor in each individual human DNA sample according to the method of Claim 32;
- (b) comparing the patterns detected in the DNA of the
30 population of humans with the patterns of a representative panel of restriction fragment length polymorphisms in a human D4 dopamine receptor gene present in humans; and

- (c) computing the frequency of each particular restriction fragment length polymorphism of a dopamine receptor gene in humans.

5 34. A method for determining the presence of a restriction fragment length polymorphism in a gene encoding a dopamine receptor in an individual human comprising the following steps:

- (a) detecting a pattern of DNA fragments of a dopamine receptor gene in the individual human according to the method of Claim 32; and
- 10 (b) comparing the pattern detected in the DNA of an individual human with the patterns of a representative panel of restriction fragment length polymorphisms in a human dopamine receptor gene.

15 35. A method for identifying a human target population for administration of a therapeutic drug for the prevention or alleviation of disease states in a human related to a human D4 dopamine receptor comprising the following steps:

- (a) detecting a pattern of DNA fragments of a human dopamine receptor gene in the individual human according to the method of Claim 32;
- 20 (b) comparing the pattern detected in the DNA of each individual human with the patterns of a representative panel of restriction fragment length polymorphisms in a human dopamine receptor gene;
- 25 (c) identifying the individual humans who are members of the target population expressing the appropriate pattern of restriction fragment length polymorphisms in a human dopamine receptor gene; and
- 30 (d) treating the members of the human target population expressing the appropriate pattern of restriction fragment length polymorphisms in the a human dopamine receptor

gene with the therapeutic drug for the prevention or alleviation of disease states in a human related to a human D4 dopamine receptor.

5 36. A reagent for detecting a restriction fragment length polymorphism in a human D4 dopamine receptor gene comprising the nucleic acid sequence of Claim 2.

37. A method for detecting alleles of a gene encoding a D4 dopamine receptor in a human comprising the following steps:

- 10 (a) isolating a sufficient quantity of DNA from the human;
- (b) amplifying *in vitro* DNA comprising a polymorphic region of the D4 dopamine receptor gene;
- (c) detecting a pattern of amplified DNA fragments of the D4 dopamine receptor gene; and
- 15 (d) identifying the alleles of the D4 dopamine receptor gene corresponding to the amplified DNA fragments detected.

38. A method for screening a population of humans to determine the frequency of alleles of a gene encoding a D4 dopamine receptor comprising the following steps:

- 20 (a) detecting a pattern of amplified DNA fragments of the D4 dopamine receptor gene in each individual human DNA sample according to the method of Claim 37;
- (b) identifying the alleles of the D4 dopamine receptor gene corresponding to the patterns of amplified DNA fragments detected in the DNA of the population of humans; and
- 25 (c) computing the frequency of each allele of the D4 dopamine receptor gene in the human population screened.

39. A method for determining a genotype of D4 dopamine receptor alleles in an individual human comprising the following steps:

- 30 (a) detecting a pattern of amplified DNA fragments of the D4 dopamine receptor gene in the individual human according to the method of Claim 37; and

- (b) identifying the alleles of the D4 dopamine receptor gene corresponding to the patterns of amplified DNA fragments detected in the DNA of the individual human.

5 40. A method for identifying a human target population for administration of a therapeutic drug for the prevention or alleviation of disease states in a human related to a human D4 dopamine receptor comprising the following steps:

- 10 (a) detecting a pattern of amplified DNA fragments of a human dopamine receptor gene in the individual human according to the method of Claim 39;
- (b) identifying the alleles comprising a genotype of the D4 dopamine receptor gene corresponding to the patterns of amplified DNA fragments detected in the DNA of the individual human;
- 15 (c) identifying the individual humans who are members of the target population having the appropriate genotype of the D4 dopamine receptor gene; and
- (d) treating the members of the human target population having the appropriate genotype of the D4 dopamine receptor gene with the therapeutic drug for the prevention or alleviation of
- 20 disease states in a human related to a human D4 dopamine receptor.

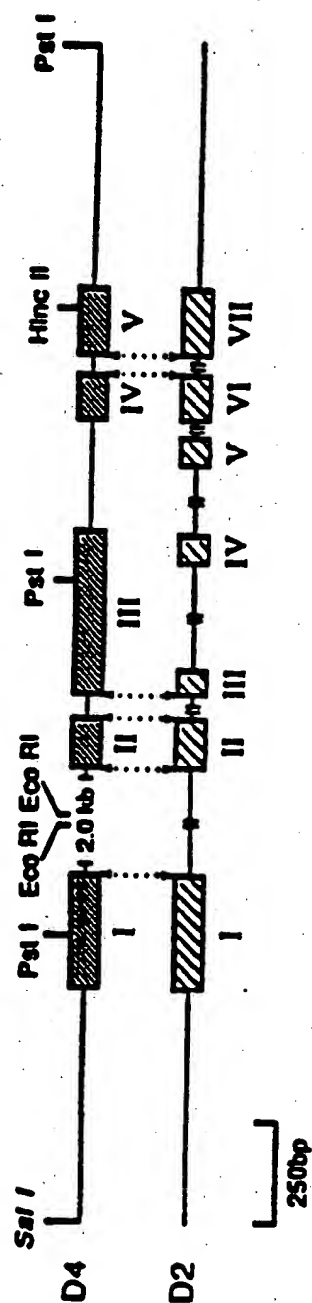


Figure 1

Figure 3

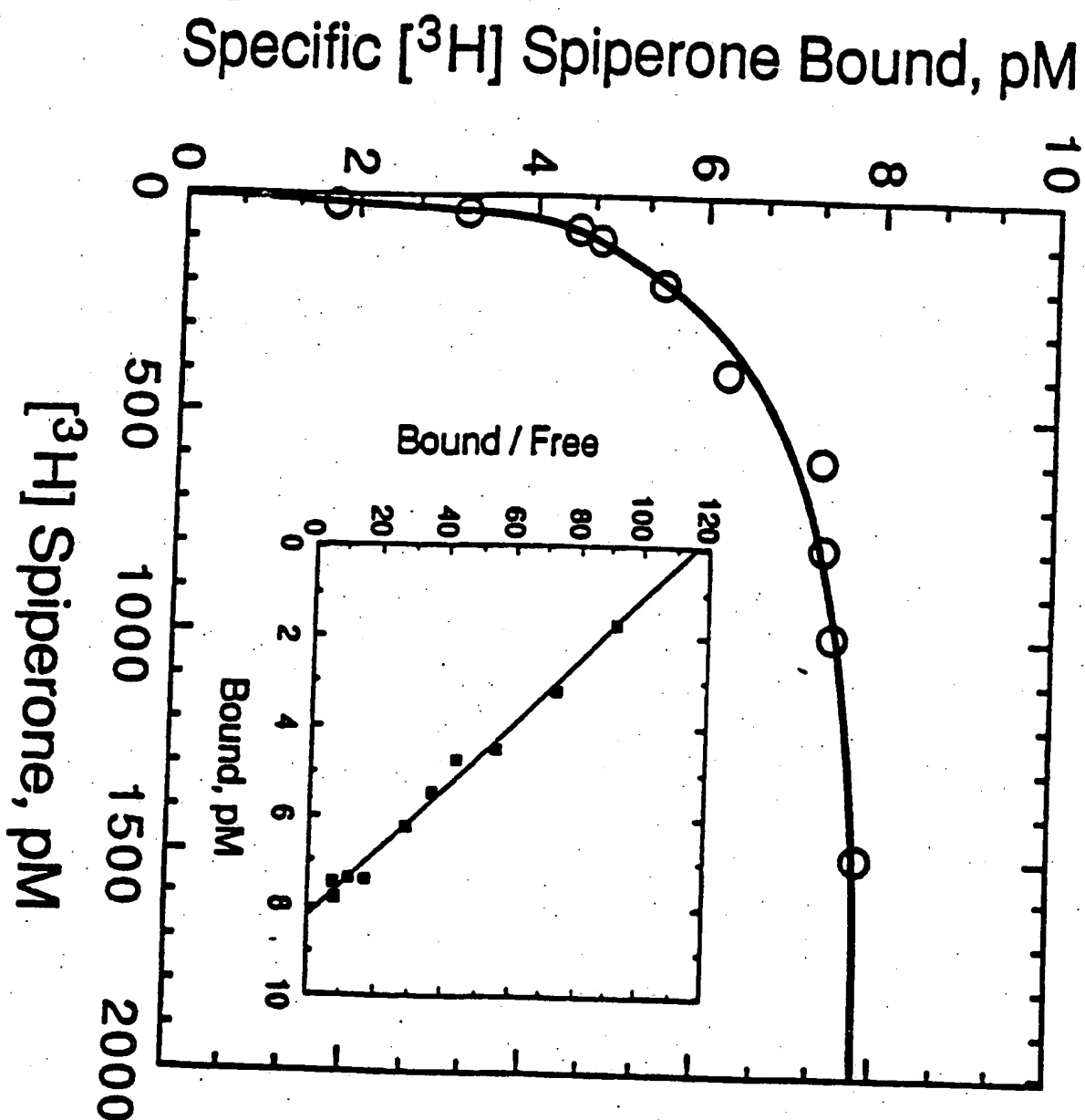


Figure 4

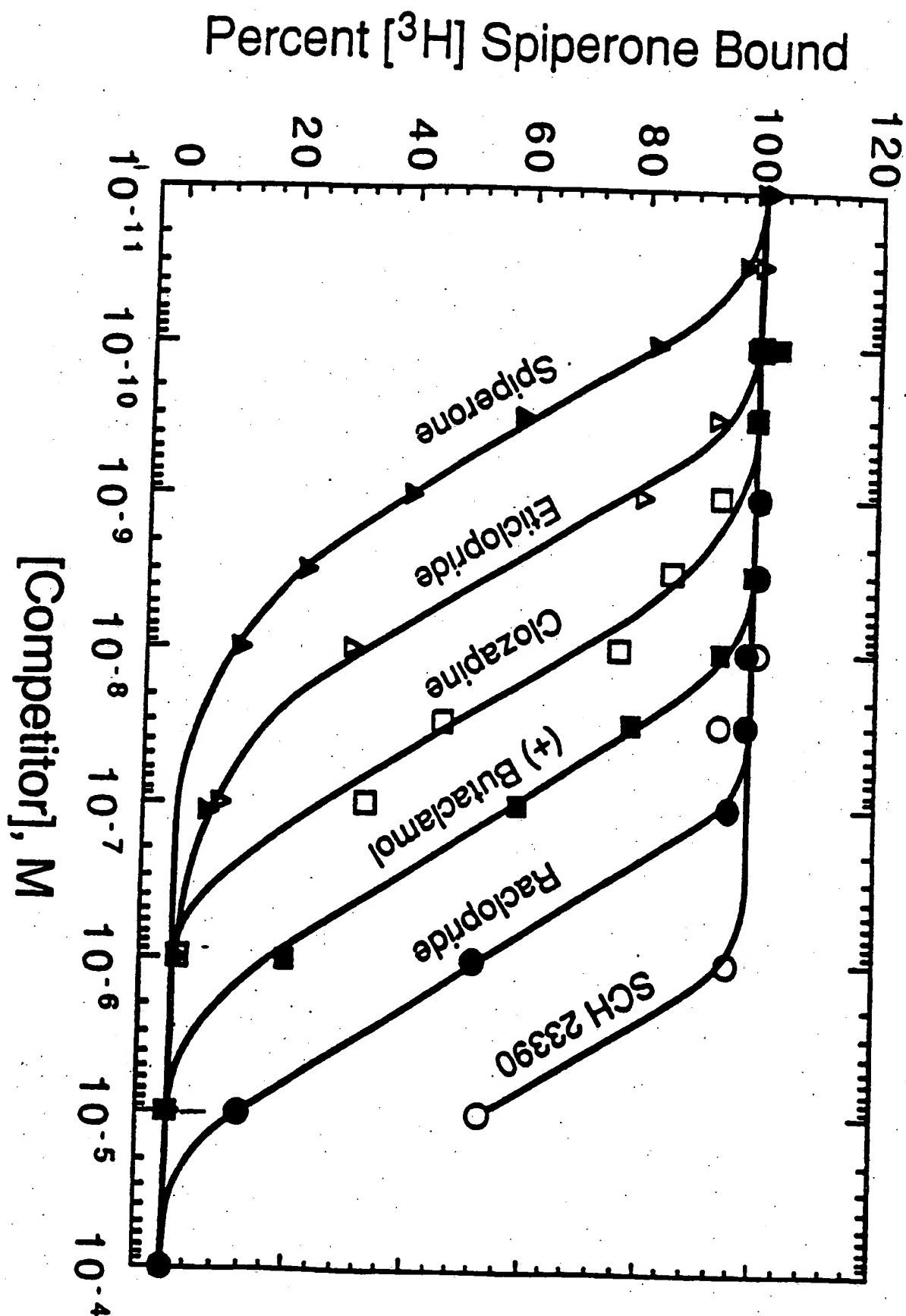
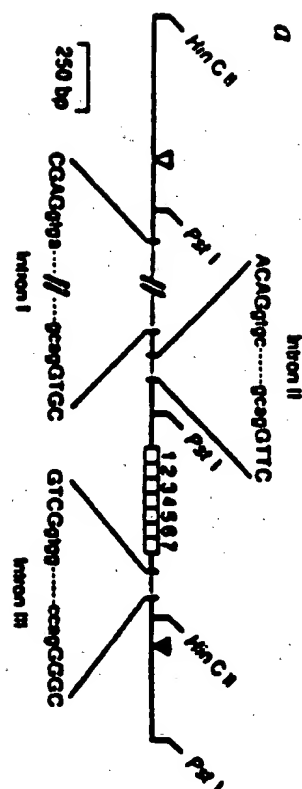


Figure 5

15



Request 1	Request 2	Request 3	Request 4	Request 5	Request 6	Request 7
<p>042 ... AC CC A CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p> <p>043 ... ACB CC A CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p> <p>047 ... AC CC A CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p>	<p>Request 2</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAT CCC TGC GGC CCC GAC TGT GCG CC</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAT CCC TGC GGC CCC GAC TGT GCG CC</p>	<p>Request 3</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p>	<p>Request 4</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p>	<p>Request 5</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAT CCC TGC GGC CCC GAC TGT GCG CC</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAT CCC TGC GGC CCC GAC TGT GCG CC</p>	<p>Request 6</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p>	<p>Request 7</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p> <p>C CCC GCG CCC CCG CTC CCC CAG GAC CCC TGC GGC CCC GAC TGT GCG CC</p>

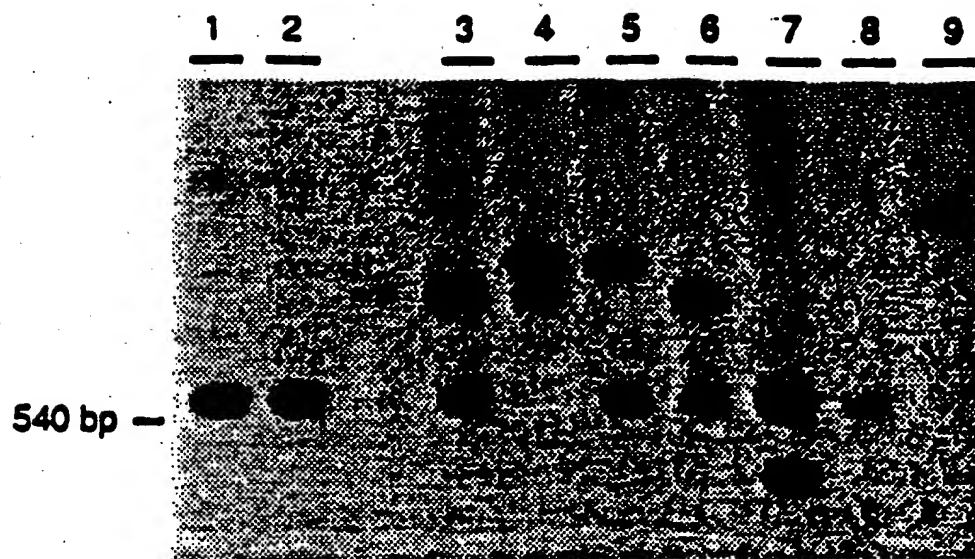


FIG 7

Fig 1

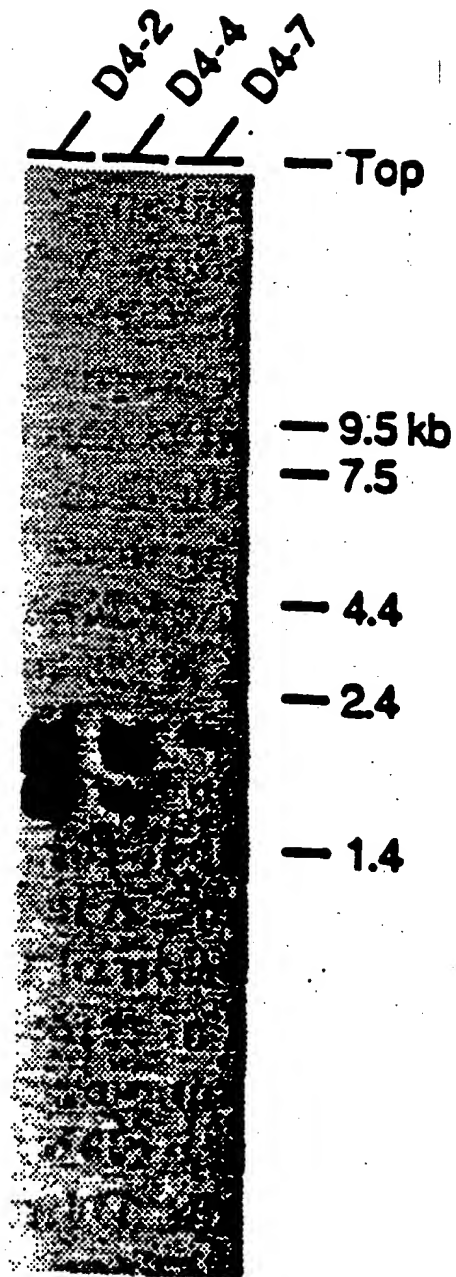


Fig 9

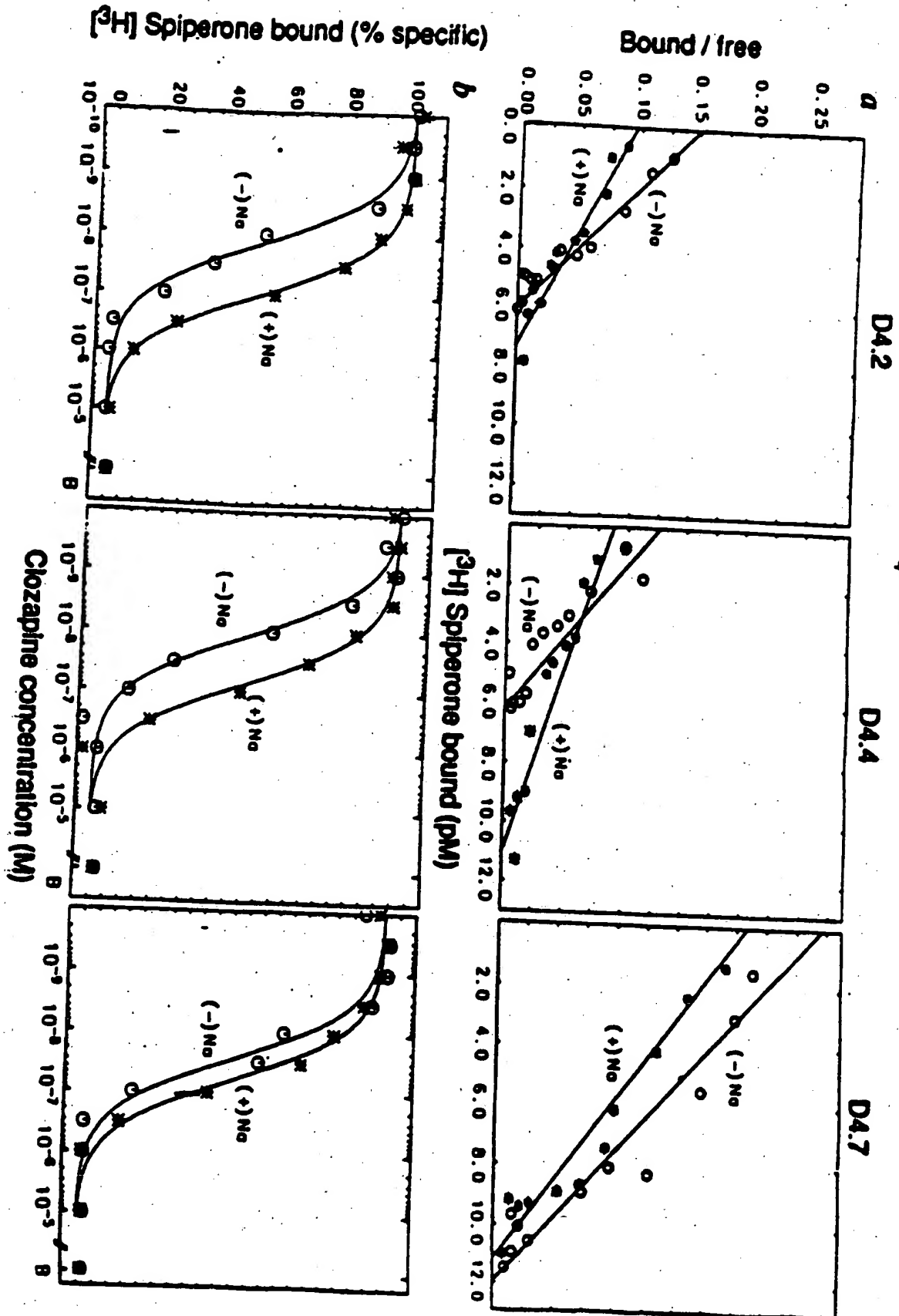


	TABLE 1 DRUG DISSOCIATION CONSTANTS (nM)			
	D ₂ (long)		D ₄	
	K _d nM	EC ₅₀	K _d nM	EC ₅₀ nM
Antagonists:				
Butaclamol(-)	0.9 M		36	0.03
Chlorpromazine	2.8 R		23	0.12
	1.5 M			0.07
Clozapine	-130 T		17	1.1
	58 R			5.1
	188 H			15.3
Eclopriide	0.08 T		0.32	0.17
Fluphenazine	0.3 T		42	0.01
Haloperidol	0.5 R		4.5	0.11
	0.8 R			0.18
	1 M			0.22
Ketanserin	182 T		147	1.31
Octoclozapine-S	1.5 T		0.6	1.38
Octoclozapine-R	13.5 T		1.8	7.17
Pimozide	2.4 R		25	0.1
Raclopride	1.8 R			0.01
	1.8 M	~1500		0.01
*Raclopride	3.2 M			0.01
Remoxipride	-300 T		2730	0.11
SCH 23390	913 M		1960	0.47
Sipiperone	0.089 R		0.08	1.15
	0.053 M			0.88
*Sipiperone	0.05 M			0.83
	0.08 M			1.5
Sutpride-S	8.2 R	~700		0.02
	4.8 R			0.08
	48 M			0.73
	15.9 M			0.25
Thiopropazine	0.21 R		53	0.004
Thionazine	3.3 R		12	0.28
Trifluoperazine	1.2 T		2.2	0.55
YM-09151-2	0.08 T		0.11	0.55
*YM-09151-2	0.09 M			0.82
Agonists:				
ADTM-(a)	H ₂ 1.7 T		H ₂ 33.7	
Apomorphine	H ₂ -2 T		H ₂ 3.3	
	24 R			
Bromocriptine	5.3 R		128	
	14 M			
Dopamine	H ₂ 7.5 T		H ₂ 18.8	
	H ₂ 2.8 T			
	474 R			
Dopamine - G	1708 R		H ₂ 48	
Ergocriptine-8	H ₂ 0.4 T		58	
Fenoldopam	H ₂ 2.8 T		420	
N-0437	H ₂ 0.7 T		83	
(-)-Noradrenaline	-4,000 T		-8000	
NPA	H ₂ 0.4 T		5.5	
PHED(-)	H ₂ 1.2 T		42	
Quinpirole(a)	578 R			
Quinpirole(-)	H ₂ 4.8 T		17	
Serotonin	-10,000 T		-8000	
SCH 38383	H ₂ 157 T		1800	
	9580 R			

Table 1. Varying concentrations of dopamine agonists and antagonists (10^{-14} - 10^{-4} M) were used to inhibit [3 H]siperone (150-200 pM) binding to membrane prepared from the COS-7 cells transfected with a 3.8 kb cDNA-gene (see text) or GH₂₃₇ cells expressing the human dopamine D₂(long) receptor. Dissociation constants were obtained by computer assisted analysis (LIGAND) as described and vary by less than 10%. *Triazole ADTM-(a); (a)-6,7-dihydroxy-2-aminotriazole; G: guanine nucleotide (e.g. Gpp(NH)p); Human: human D₂(long); NPA: N-propylnorapomorphine; N-0437: 2-(4-n-propyl-N-phenylethylamino)-6-hydroxytryptamine.HCl; p: present study, using GH₂₃₇ cells and [3 H]siperone; Quinpirole(-): LY171558; R: Rat D₂(long); T: Ki in striatum or pg in rat pituitary tissue homogenate

INTERNATIONAL SEARCH REPORT

In International Application No
PCT/US 93/07370

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/12 C07K13/00 G01N33/68 C12N5/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N C07K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 10571 (STATE OF OREGON) 25 June 1992 see the whole document ---	1-31,36
X	NATURE vol. 358 , 9 July 1992 , LONDON GB pages 149 - 152 H.H.M. VAN TOL ET AL. 'Multiple Dopamine D4 receptor variants in the human population' see the whole document ---	1-34, 36-39
A	WO,A,91 12339 (BOARD OF REGENTS, UNIVERSITY OF TEXAS SYSTEM ET AL.) 22 August 1991 --- -/--	32-34, 36-39

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

14 January 1994

Date of mailing of the international search report

01-02-1994

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Authorized officer

VAN PUTTEN, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/07370

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUCLEIC ACIDS RESEARCH. vol. 19, no. 20, 25 October 1991, EYNHAM, OXFORD ENGLAND page 5801 J.L. KENNEDY ET AL. 'A HincII RFLP in the human D4 dopamine receptor locus (DRD4)' ----	32-34, 36-39
A	NATURE vol. 350, 18 April 1991, LONDON GB pages 610 - 614 H.H.M. VAN TOL ET AL. 'Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine' -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/07370

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 35, 40 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/07370

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9210571	25-06-92	AU-A- 9140391 EP-A- 0574406	08-07-92 22-12-93
WO-A-9112339	22-08-91	EP-A- 0514490 US-A- 5210016	25-11-92 11-05-93